

角蛋白? ‘ 艾尤蓄莖尿z選及酵素分析與應用

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摘要

從彰化養雞場的羽毛廢棄物土壤中，篩選出六株初步命名為Wu1、Wu2、Wu3、Wu4、Wu5及Wu6之具分泌角蛋白?能力的羽毛降解微生物，經由微生物菌種鑑定並命名為*Bacillus megaterium* Wu1、*Bacillus cereus* Wu2、*Bacillus cereus* Wu3、*Brevibacillus parabrevis* Wu4、*Bacillus thuringiensis* Wu5及*Bacillus cereus* Wu6。除了Wu3之外，其餘五株菌在培養基初始酸鹼值為pH 5.0時，有最大角蛋白?活性，Wu3則是pH 9.0。六株菌最適的生長溫度範圍在30-40°C之間。*B. megaterium* Wu1是唯一不需額外添加碳氮源，就具有較高的角蛋白?活性。將家禽廢棄羽毛作為六株菌生長時的碳源與氮源，均可產生胞外角蛋白?。將發酵液經過硫酸銨沉澱、Sephacryl S-200 HR膠體層析及DEAE Sephadex A-50離子交換樹脂的純化，角蛋白?Wu1、Wu3、Wu5及Wu6的純化倍率分別為7.63、19.48、2.23及4.71，合成率分別為13.59%、26.32%、16.60%及10.55%。以SDS-PAGE分析純化後酵素的分子量，分別為34、46、32及55與68 kDa，其中角蛋白?Wu6為二聚體。以偶氮酪蛋白為基質，*B. megaterium* Wu1角蛋白?於pH 4-12.0與溫度範圍為10-100°C下有活性，最適pH與溫度分別為pH 7與50°C。*B. cereus* Wu6角蛋白?於pH 6.0-11.0，10-100°C範圍內具有活性，最適pH與溫度分別為pH 8與50°C。金屬螯合劑EDTA和O-phenanthroline蛋白?抑制劑會對本研究角蛋白?之酵素活性造成抑制，因此此二種角蛋白?皆金屬型蛋白?。發現於*B. megaterium* Wu1和*B. cereus* Wu6角蛋白?中添加Na⁺和Mg²⁺離子可增加酵素活性。*B. megaterium* Wu1和*B. cereus* Wu6角蛋白?以粉末形式貯存於最具有穩定性，若以液態形式存在於室溫下，酵素會快速失活。此外，添加一些有機溶劑對Wu1可穩定酵素活性；添加還原劑則會對*B. megaterium* Wu1和*B. cereus* Wu6的活性造成抑制效果。特別是*B. megaterium* Wu1和*B. cereus* Wu6角蛋白?以偶氮酪蛋白為基質，Wu1和Wu6角蛋白?之Km值，分別為0.85和3.28 g/L。

關鍵詞：角蛋白?、B羽毛廢棄物、金屬蛋白?

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參考文獻

- 1.吳芝穎。2004。 *Bacillus licheniformis* THSC-1 角蛋白分解之純化、定性與基因選殖:第1-88頁。東海大學碩士論文。臺中，臺灣。
- 2.宋思揚、樓士林。2002。生物技術概論。第135-141頁。滄海書局。臺中，臺灣。
- 3.李京樺。2006。角蛋白生產菌篩選、純化及其特性:第1-32頁。臺灣海洋大學碩士論文。基隆，臺灣。
- 4.沈潔瑩。2008。篩選角蛋白生產菌及其酵素性質研究:第1-82頁。屏東科技大學碩士論文。屏東，臺灣。
- 5.張資奇。1999。蛋雞糞堆肥中篩選雞毛分解菌之研究:第1-102頁。東海大學碩士論文。臺中，臺灣。
- 6.莊榮輝。2000。酵素化學實驗。國立台灣大學農業化學系生物化學研究室。臺北，臺灣。
- 7.陳庭柔。2004。 *Bacillus licheniformis* 14353 和 *Bacillus licheniformis* 11594 之粗酵素對雞羽毛水解效果之評估:第1-89頁。中興大學碩士論文。臺中，臺灣。
- 8.陳瑩和王宇新。2002。角蛋白及其提取。材料導報 16 (12): 65-67。
- 9.黃如婕。2008。豬毛篩選菌 *Bacillus cereus* H10 角蛋白及蛋白質純化、特性與應用之研究:第1-63頁。臺灣大學碩士論文。臺北，臺灣。
- 10.賈如琰、何玉鳳、王榮民、李芙蓉、王艷。2008。角蛋白的分子構成、提取及應用。化學通報 71 (4):1-6。
- 11.趙曉芳。2002。羽毛粉的加工利用。廣東飼料11:13-14。
- 12.劉曉霞和郭曉輝。2001。膨化羽毛粉飼餵肉鴨試驗。獸醫與飼料添加劑 2:5-6。
- 13.謝魁鵬、魏耀輝。1985。最新生物化學實驗。藝軒出版社。台北，台灣。
- 14.蘇夢蘭。2000。化腐朽為神奇-家禽屠宰副產物化製場。農政與農情100:17-20。
- 15.蘇睿綺。2006。枯草菌屬角蛋白之純化與性質研究:第1-132頁。靜宜大學食品營養學研究所碩士論文。臺中，臺灣。
- 16.Abdel-Hafez, A. I. I. and El-Sharoumy, H. M. M. 1990. The occurrence of keratinolytic fungi in sewage sludge from Egypt. J. Basic Microbiol. 30: 73-79.
- 17.Adbul-Fatah, H. M., Moubasher, H. H. and Maghazy, S. M. 1982. Keratinolytic fungi in Egyptian soils. Mycopathologia. 79: 49-53.
- 18.Akhtar, W. and Edwards, H. G. M. 1997. Fourier-transform raman spectroscopy of mammalian and avian keratotic biopolymers. Spectrochim acta part A: molecular and biomolecular spectroscopy 53: 81-90.
- 19.AI Musallam, A. A. and Radwan, S. S. 1990. Wool colonizing microorganisms capable of utilizing wool lipids and fatty acids as sole sources of carbon and energy. J. Appl. Bacteriol. 69: 806-813.
- 20.Allpress, J. D., Mountain, G. and Gowland, P. C. 2002. Production, purification and characterization of an extracellular keratinase from *Lysobacter* NCIMB 9497. Lett. Appl. Microbiol. 34: 337-342.
- 21.AI-Musallam, A. A. 1988. Distribution of keratinolytic fungi in the desert soil of Kuwait. Mycoses. 32: 296-302.
- 22.AI-Musallam, A. A. 1990. Distribution of keratinolytic fungi in animal folds in Kuwait. Mycopathologia. 79: 49-53.
- 23.AI-Musallam, A. A., Alzarban, S. S., Al-Sane, N. A. and Ahmad, T. M. 1995. A report on the predominance of a dermatophyte species in cultivated soil from Kuwait. Mycopathologia. 130: 159-161.
- 24.Anbu, P., Gopinath, S. C. B., Hilda, A., Lakshmi priya, T. and Annadurai, G. 2005. Purification of keratinase from poultry farm isolate-*Scopulariopsis brevicaulis* and statistical optimization of enzyme activity. Enzyme Microb. Technol. 36: 639-647.
- 25.Anbu, P., Gopinath, S. C. B., Hilda, A., Lakshmi priya, T. and Annadurai, G. 2007. Optimization of extracellular keratinase production by poultry farm isolate *Scopulariopsis brevicaulis*. Bioresour. Technol. 98: 1298 – 1303.
- 26.Apodaca, G. and Mckerrow, J. H. 1989. Regulation of *Trichophyton rubrum* proteolytic activity. Infect. Immun. 57: 3081-3090.
- 27.Asahi, M. R., Lindquist, K., Fukuyama, G., Apocarda, W. L., Epstein, W. L. and McKerrow, J. H. 1985. Purification and characterization of major extracellular proteinases from *Trichophyton rubrum*. Biochem. J. 232: 139-144.
- 28.Ashour, S. A., El-Shorah, H. M. and Ghanem, A. A. 1992. Keratinolytic activity of thermophilic bacteria isolated from Egyptian soil. J. Environ. Sci. 4: 335-339.
- 29.Bahuguna, S. and Kushwaha, R. K. S. 1989. Hair perforation by keratinophilic fungi. Mycoses. 32: 340-343.
- 30.Bajorath, J., Hinrichs, W. and Saenger, W. 1988. The enzymatic activity of proteinase K is controlled by calcium. Eur. J. Biochem. 176: 441-447.
- 31.Balaji, S., Senthil Kumar, M., Karthikeyan, R. Kumar, R., Kirubanandan, S., Sridhar, R. and Sehgal, P. K. 2008. Purification and characterization of an extracellular keratinase from a hornmeal-degrading *Bacillus subtilis* MTCC (9102). World J. Microbiol. Biotechnol. 24: 2741-2745.
- 32.Banerjee, U. C., Sani, R. K., Azmi, W. and Soni, R. 1999. Thermostable alkaline protease from *Bacillus brevis* and its characterization as a laundry detergent additive. Process Biochem. 35: 213 – 219.
- 33.Barett, A. J. 1994. Proteolytic enzymes: serine and cysteine peptidases. Methods enzymol. 244: 1-15.
- 34.Baxter, M. and Mann, P. R. 1969. Electron microscopic studies on the invasion of human hair in vitro by three keratinophilic fungi. Sabouraudia. 7: 33-37.
- 35.Benedek, A., Szabo, I., Barabas, G., Czappan, M. and Szabo, G. 1985. Digestion of chicken feather by keratinase enzyme(s) of an Actinomycetes strain. In: Biological, Biochemical and Biomedical Aspects of Actinomycetes. Proceedings of the Sixth International Symposium on Actinomycete Biology, Debrecen, Hungary, 26-30 August, 1985, ed. G. Szabo, S. Biro and M. Goodfellow, Akademiai Kiado, Budapest, 1986.
- 36.Bernal, C., Cairo, J. and Coello, N. 2006. Purification and characterization of a novel exocellular keratinase from *Kocuria rosea*. Enzyme Microb. Technol. 38: 49-54.
- 37.Bernal, C., Vidal, L., Valdivieso, E. and Coello, N. 2003. Keratinolytic activity of *Kocuria rosea*. World J. Microbiol. Biotechnol. 19: 255-261.
- 38.Bertsch, A. and Coello, N. 2005. A biotechnological process for treatment and recycling poultry feathers as a feed ingredient. Bioresour. Technol. 96: 1703-1708.
- 39.Bhaskar, N., Sudeepa, E. S., Rashmi, H. N. and Selvi, A. 2007. Partial purification and characterization of protease of *Bacillus proteolyticus* CFR3001 isolated from fish processing waste and its antibacterial activities. Bioresour. Technol. 98: 2758-2764.
- 40.Bockle, B., Galunsky, B. and Muller, R. 1995. Characterization of a keratinolytic serine proteinase from *Streptomyces pactum* DSM 40530. Appl. Environ.

Microbiol. 61: 3705-3710. 41. Boguslawski, G., Shultz, J. L. and Yehle, C. O. 1983. Purification and characterization of an extracellular protease from *Flavobacterium arborescens*. *Anal. Biochem.* 132: 41-49. 42. Bradbury, J. H. 1973. The structure and chemistry of keratin fibers. *Adv Prot Chem.* 67: 111-211. 43. Bressollier, P., Letourneau, F., Urdaci, M., and Verneuil, B. 1999. Purification and characterization of a keratinolytic serine proteinase from *Streptomyces albidoflavus*. *Appl. Environ. Microbiol.* 65: 2570-2576. 44. Burgess, A. W., Weinstein, L. I., Gabel, D. and Scheraga, H.A. 1975. Immobilized carboxypeptidase A as a probe for studying the thermally induced unfolding of bovine pancreatic ribonuclease. *Biochemistry* 28: 5421-5428. 45. Cai, C. G., Lou, B. G. and Zheng, X. D. 2008. Keratinase production and keratin degradation by a mutant strain of *Bacillus subtilis*. *J Zhejiang Univ Sci B.* 9: 60-67. 46. Carter, D. D. and Shih, J. C. H. 1997. In vitro and in vivo studies of the effect of keratinase on the digestibility of commercial feather meal and other proteins. *Poultry Sci.* 76: 1-22. 47. Chandrasekaran, S. and Dhar, S. C. 1986. Utilization of multiple proteinase concentrate to improve the nutritive value of chicken feather meal. *J. Leather Res.* 4: 23-30. 48. Chapman, J. D. and Hultin, H. O. 1975. Some properties of a protease (subtilisin BPN[®]) immobilized to porous glass. *Biotechnol Bioeng.* 17: 1783-1795. 49. Chen, S. X., Swaisgood, H. E. and Foegeding, E. A. 1994. Gelation of β -lactoglobulin treated with limited proteolysis by immobilization trypsin. *J. Agric. Food Chem.* 42: 234-239. 50. Chen, S. Y., Hardin, C. C., Swaisgood, H. E. 1993. Purification and characterization of β -structural domains of β -lactoglobulin liberated by immobilized proteolysis. *J. Protein Chem.* 12: 613-625. 51. Cheng, S. W., Hu, H. M., Shen, S. W., Takagi, H., Asano, M. and Tsai, Y. C. 1995. Production and characterization of keratinase of a feather degrading *Bacillus licheniformis* PWD-1. *Biosci. Biotechnol. Biochem.* 59: 2239-2243. 52. Chitte, R. R., Nalawade, V. K. and Dey, S. 1999. Keratinolytic activity from the broth of a feather-degrading thermophilic *Streptomyces thermoviolaceus* strain SD8. *Lett Appl Microbiol.* 28: 131-136. 53. Choi, J. M. and Nelson, P. V. 1996. Developing a slow-release nitrogen fertilizer from organic sources II: using poultry feathers. *J. Am. Soc. Hortic. Sci.* 121: 634-638. 54. Church, F. C., Catiagnani, G. L. and Swaisgood, H. E. 1982. Use of immobilized *Streptomyces griseus* protease (pronase) as a probe of structural transitions of lysozyme, β -lactoglobulin and casein. *Enzyme Microb. Technol.* 4: 317-321. 55. Church, F. C., Swaisgood, H. E. and Catiagnani, G. L. 1984. Compositional analysis of proteins following hydrolysis by immobilized proteinases. *J Appl. Biochem.* 6: 205-6211. 56. Cortezi, M., Cilli, E. M., Contiero, J. 2008. *Bacillus amyloliquefaciens*: A new keratinolytic feather-degrading bacteria. *Current Trends in Biotechnology and Pharmacy* 2: 170-177. 57. Coward-Kelly G., Chang, V. S., Agbogbo F. K. and Holtzapple, M. T. 2006. Lime treatment of keratinous materials for the generation of highly digestible animal feed: 1. Chicken feathers. *Bioresour. Technol.* 97: 1337-1343. 58. Dalev, P., Ivanov, I. and Liubomirova, A. 1997. Enzymic modification of feather keratin hydrolysates with lysine aimed at increasing the biological value. *J. Sci. Food. Agric.* 73: 242-244. 59. de Groot, A. P. and Slump, P. 1969. Effects of severe alkali treatment of proteins on amino acid composition and nutritive value. *J. Nutr.* 98: 45-56. 60. Desmukh, S. K. and Agrawal, S. C. 1982. In vitro degradation of human hair by some keratinophilic fungi. *Mykosen.* 25: 454-458. 61. Desmukh, S. K. and Agrawal, S. C. 1985. Degradation of human hair by some dermatophytes and other keratinophilic fungi. *Mykosen.* 28: 463-466. 62. Deydier, E., Guilet, R., Sarda, S. and Sharrock, P. 2005. Physical and chemical characterization of crude meat and bone meal combustion residue: "waste or raw material?" *J. Hazard. Mater.* 121: 141-148. 63. Dix, N. J. and Webster, J. 1995. Fungal Ecology. Chapman and Hall, London. 64. Dozie, I. N. S., Okeke, C. N. and Unaeze, N. C. 1994. A thermostable, alkaline-active, keratinolytic proteinase from *Chrysosporium keratinophilum*. *World J. Microbiol. Biotechnol.* 10: 563-567. 65. Ebeling, W. N., Hennrick, M., Klockow, H., Metz, H., Orth, D. and Lang, H. 1974. Proteinase K from *Titirachium album* Limber. *Eur. J. Biochem.* 47: 91-97. 66. Eggum, B. O. 1970. Evaluation of protein quality of feather meal under different treatments. *Acta Agric. Scand.* 20: 230-234. 67. Elmayergi, H. H. and Smith, R. E. 1971. Influence of growth of *Streptomyces fradiae* on pepsin-HCl digestibility and methionine content of feather meal. *Can. J. Microbiol.* 17: 1067-1072. 68. El-Naghy, M. A., El-Ktatny, M. S., Fadl-Allah, E. M. and Nazeer, W. W. 1998. Degradation of chicken feathers by *Chrysosporium georgiae*. *Mycopathologia* 143: 77-84. 69. El-Refai, H. A., AbdelNaby, M. A., Gaballa, A., El-Araby M. H. and Abdel Fattah, A.F. 2005. Improvement of the newly isolated *Bacillus pumilus* FH9 keratinolytic activity. *Process Biochemistry.* 40: 2325-2332. 70. El-Shora, H. M., Ashour, S. A. and Ghanem, A. A. 1992. Growth and keratinolytic activity of selected *Bacillus* spp. in relation to the initial pH, molarity, different vitamins and trace elements. *Egypt. J. Appl. Sci.* 7: 320-334. 71. Evans, K. L., Crowder, J. and Miller, E. S. 2000. Subtilisins of *Bacillus* spp. hydrolyze keratin and allow growth on feathers. *Can. J. Microbiol.* 46: 1004-1011. 72. Fakhfakh-Zouari, N., Haddar, A., Hmidet, N., Frikha, F. and Nasri, M. 2010. Application of statistical experimental design for optimization of keratinases production by *Bacillus pumilus* A1 grown on chicken feather and some biochemical properties. *Process biochemistry.* 45: 617-626. 73. Farag, A. M. and Hassan, M. A. 2004. Purification, characterization and immobilization of a keratinase from *Aspergillus oryzae*. *Enzyme Microb. Technol.* 34: 85-93. 74. Fasasi, Y. A. 1997. Studies on keratinophilic actinomycetes isolated from Kuwait soil. MSc Thesis, Department of Biological Sciences, Kuwait University. 75. Feder, J., Garrett, L. R., and Wildi, B. S. 1971. Studies on the role of calcium in thermolysin. *Biochemistry* 10: 4552-4556. 76. Figueras, M. J., Gurrado, J. and Zaror, L. 1997. Ultrastructural aspects of hair digestion in black piedra infection. *J. Med. Vet. Mycol.* 35: 1-6. 77. Fox, P. F., Power, P., and Cogan, T. M. 1989. Isolation and molecular characteristics, in "Enzymes of Psychrotrophs in Raw Food". p. 57-120. CRC Press, Boca Raton, USA. 78. Friedrich, A. B. and Antranikian, G. 1996. Keratin degradation by *Frevidobacterium pennavorans*, a novel thermophilic anaerobic species of the order thermotogales. *Appl. Environ. Microbiol.* 62: 2875-2882. 79. Friedrich, J. and Kern, S. 2003. Hydrolysis of native proteins by keratinolytic protease of *Doratomyces microsporus*. *J. Mol. Catal., B Enzym.* 21: 35-37. 80. Garcia de Fernando, G. D., and Fox, P. F. 1991. Extracellular proteinases from micrococcus GF. 1. Factors affecting growth and production. *Lait.* 71: 371-382. 81. Garrett, R. H. and Grisham, C. M. 2002. Principles of biochemistry: with a human focus. p.128-129. Fort worth: Harcourt college pub, Belmont, USA. 82. Gassesse, A., Kaul, R. H., Gashe, B. A. and Mattiasson, B. 2003. Novel alkaline proteases from alkalophilic bacteria grown on chicken feather. *Enzyme Microb. Technol.* 32: 519-524.

83.Ghorbel, B., Sellami-Kamoun, A. and Nasri, M. 2003. Stability studies of protease from *Bacillus cereus* BG1. *Enzyme Microb. Technol.* 32: 513-518. 84.Ghosh, A., Chakrabarti, K. and Chattopadhyay, D. 2008. Degradation of raw feather by a novel high molecular weight extracellular protease from newly isolated *Bacillus cereus* DCUW. *J. Ind. Microbiol. Biotechnol.* 35: 825-834. 85.Gousterova, A., Braikova, D., Goshev, I., Christov, P., Tishinov, K., Tonkova, V. E., Haertle, T. and Nedkov, P. 2005. Degradation of keratin and collagen containing wastes by newly isolated thermoactinomycetes or by alkaline hydrolysis. *Lett. Appl. Microbiol.* 40: 335-340. 86.Govind, N. S., Merta, B., Sharma, M. and Modi, V. V. 1981. Protease and carotenogenesis in *Blakeslea trispora*. *Phytochemistry* 20: 2483-2485. 87.Gradisar, H., Friedrich, J., Krizaj, I. and Jerala, R. 2005. Similarities and specificities of fungal keratinolytic proteases: comparison of keratinases of *Paecilomyces marquandii* and *Doratomyces microsporus* to some known proteases. *Appl. Environ. Microbiol.* 71: 3420-3426. 88.Gradisar, H., Kern, S. and Friedrich, J. 2000. Keratinase of *Doratomyces microsporus*. *Appl. Microbiol. Biotechnol.* 53: 196-200. 89.Grappel, S. F. and Blank, F. 1972. Role of keratinase in dermatophyte. *Dermatologica.* 145: 245-255. 90.Grimwood, B. G., Hechemy, K. and Stevens, R. W. 1994. Purification and characterization of a neutral zinc endopeptidase secreted by *Flavobacterium meningosepticum*. *Arch. Biochem. Biophys.* 311: 127-132. 91.Gripon, J. C., Auberger, B. and Lenoir, J. 1980. Metalloproteases from *Penicillium caseicolum* and *P. roqueforti*: comparison of specificity and chemical characterization. *Int. J. Biochem.* 12: 451-455. 92.Gupta, M. N. 1992. Enzyme function in organic solvents. *Eur. J. Biochem.* 203: 25-32. 93.Gupta, R. and Ramnani, P. 2006. Microbial keratinases and their prospective applications: an overview. *Appl. Microbiol. Biotechnol.* 70: 21-33. 94.Gupta, R., Beg, Q. K. and Lorenz, P. 2002. Bacterial alkaline proteases: molecular approaches and industrial applications. *Appl. Microbiol. Biotechnol.* 59: 15-32. 95.Hadas, A. and Kautsky, L. 1994. Feather meal, a semi-slow-release nitrogen fertilizer for organic farming. *Nutr. Cycl. Agroecosyst.* 38: 165-170. 96.Hanel, H., Kalisch, J., Keil, M., Marsch, W. C. and Buslau, M. 1991. Quantification of keratinolytic activity from *Dermatophilus congolensis*. *Med. Microbiol. Immun.* 180: 45-51. 97.Haruta, S., Nakayama, T., Nakamura, K., Hemmi, H., Ishii, M., Igarashi, Y. and Nishino, T. 2005. Microbial diversity in biodegradation and reutilization processes of garbage. *J. Biosci. Bioeng.* 99: 1-11. 98.Higuchi, D., Takiuchi, J. and Negi, M. 1981. The effect of keratinase on human epidermis especially on stratum corneum. *Jpn. J. Dermatol.* 91: 119-125. 99.Hirchman, D. J., Zametkin, J. M. and Rogers, R. E. 1994. The utilization of wool by four saprophytic microorganisms in the presence of added nutrients. *Am. Dyestuff Rep.* 33: 353-359. 100.Holmquist, B. and Vallee, B. L., 1974. Metal substitutions and inhibition of thermolysin: spectra of the cobalt enzyme. *J. Biol. Chem.* 249: 4601-4607. 101.Ignatova, Z., Gousterova, A., Spassov, G. and Nedkov, P. 1999. Isolation and partial characterization of extracellular keratinase from a wool degrading thermophilic actinomycete strain *Thermoactinomyces candidus*. *Can. J. Microbiol.* 45: 217-222. 102.Ionata, E., Canganella, F., Bianconi, G., Benno, Y., Sakamoto, M., Capasso, A., Rossi, M. and La Cara F. 2008. A novel keratinase from *Clostridium sporogenes* bv. *pennavorans* bv. nov., a thermotolerant organism isolated from solfataric muds. *Microbiological Research.* 163: 105-112. 103.Juan, S. M., and Cazzulo, J. J. 1976. The extracellular protease from *Pseudomonas fluorescens*. *Experientia.* 32: 1120-1122. 104.Kembhavi, A. A., Kulharni A. and Pant, A. A. 1993. Salt-tolerant and thermostable alkaline protease from *Bacillus subtilis* NCIM No. 64. *Appl. Biochem. Biotechnol.* 38: 83-92. 105.Khan, M. R., Blain, J. A. and Patterson, J. D. E. 1983. Partial purification of *Mucor pusillus* intracellular proteases. *Appl. Environ. Microbiol.* 45: 94-96. 106.Kim, J. M., Lim, W. J. and Suh, H. J. 2001. Feather-degrading *Bacillus* species from poultry waste. *Process Biochem.* 37: 287-291. 107.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: 787-797. 108.Kim, W. K. and Patterson P. H. 2000. Nutritional value of enzyme- or sodium hydroxide-treated feathers from dead hens. *Poultry Sci.* 79: 528-34. 109.Kitadokoro, K., Tsuzuki, H., Nakamura, E., Sato, T. and Teraoka, H. 1994. Purification and characterization, primary structure, crystallization and preliminary crystallographic study of a serine proteinase from *Streptomyces fradiae* ATCC 14544. *Eur. J. Biochem.* 220: 55-61. 110.Kojima, M., Kanai, M., Tominaga, M., Kitazume, S., Inoue, A. and Horikoshi, K. 2006. Isolation and characterization of a feather-degrading enzyme from *Bacillus pseudofirmus* FA30-01. *Extremophiles* 10: 229-235. 111.Kumar, C. G. 2002. Purification and characterization of a thermostable alkaline protease from alkalophilic *Bacillus pumilus*. *Lett. Appl. Microbiol.* 34: 13-17. 112.Kunert, J. 1972. Keratin decomposition by dermatophytes: evidence of sulphitolysis of the protein. *Experientia.* 28: 1025-1026. 113.Kunert, J. 1973. Keratin decomposition by dermatophytes: 1. Sulfite production as a possible way of substrate denaturation. *Zeitschrift fuer Allgermerine Microbiologie Morphologie, Genetic und Oekologie der Microorganismen.* 13: 489-498. 114.Kunert, J. 1975. Formation of sulphate, sulfite and S-sulfocysteine by the fungus *Microsporium gypseum* during growth on cystine. *Folia Microbiologica* 20: 142-151. 115.Kunert, J. 1976. Keratin decomposition by dermatophytes: II. Presence of S-sulfocysteine and cysteic acid in soluble decomposition product *Zeitschrift fuer Allgermerine Microbiologie Morphologie, Genetic und Oekologie der Microorganismen.* 16: 97-105. 116.Kunert, J. 1985a. Metabolism of sulphur containing amino acids in the dermatophyte *Microsporium gypseum*. I: Neutral amino acids. *J. Basic. Microbiol.* 25: 31-37. 117.Kunert, J. 1985b. Metabolism of sulphur containing amino acids in the dermatophyte *Microsporium gypseum*. II: Acidic amino acids derivatives. *J. Basic. Microbiol.* 25: 111-118. 118.Kunert, J. 1987. Utilization of various concentrations of free cystine by fungus *Microsporium gypseum*. *J. Basic. Microbiol.* 27: 207-213. 119.Kunert, J. 1988. Thiosulfate production from cystine by the keratinolytic prokaryote *Streptomyces fradiae*. *Arch. Microbiol.* 150: 600-601. 120.Kunert, J. 1989. Biochemical mechanisms of keratin degradation by actinomycete *Streptomyces fradiae* and fungus *Microsporium gypseum*, a comparison. *J. Basic Microbiol.* 29: 597-604. 121.Kunert, J. 1992. Effects of reducing agents on proteolytic and keratinolytic activity of enzymes of *Microsporium gypseum*. *Mycoses* 35: 343-348. 122.Kunert, J. and Krajci, D. 1981. An electron microscopy study of keratin degradation by the fungus *Microsporium gypseum* in vitro. *Mykosen* 24: 485-496. 123.Kunert, J. and Stransky, Z. 1988. Thiosulfate production from cysteine by the keratinophilic prokaryote *Streptomyces fradiae*. *Arch. Microbiol.* 150: 600-01. 124.Kunert, J., 2000. Physiology of keratinophilic fungi. In: Kushwaha, R.K.S., Guarro, J. (Eds.), *Biology of Dermatophytes and Other*

Keratinophilic Fungi, Revista Iberoamericana de Micologia, pp. 77-85. Bilbao. 125. Kunitate, A., Okamoto, M. and Ohmori, I. 1989. Purification and characterization of a thermostable serine protease from *Bacillus thuringiensis*. *Agric. Biol. Chem.* 53: 3251-3256. 126. Kushwaha, R. K. S. 1983. The in vitro degradation of peacock feathers by some fungi. *Mykosen* 26: 324-326. 127. Lamkin, I., Hamilton, A. J. and Hay, R. J. 1996. Purification and characterisation of a novel 34,000-Mr cell-associated proteinase from the dermatophyte *Trichophyton rubrum*. *FEMS Immunol. Med. Microbiol.* 13: 131-140. 128. Langeveld, J. P. M., Wang, J. J., Van de Wiel, D. F. M., Shih, G. C., Garssen, G. J., Bossers, A. and Shih, J. C. H. 2003. Enzymatic degradation of prion protein in brain stem from infected cattle and sheep. *J. Infect. Dis.* 188: 1782-1789. 129. Larcher, G., Cimon, B., Symoens, F., Tronchin, G., Chabasse, D. and Bouchara J. P. 1996. A 33 kDa serine proteinase from *Scedopodium apiospermum*. *Biochem. J.* 315: 119-126. 130. Larsen, K. S. and Auld, D. S. 1989. Carboxypeptidase A: mechanism of zinc inhibition. *Biochemistry.* 28: 9620-9625. 131. Latshaw, J. D., Musharaf, N. and Retrum, R. 1994. Processing of feather meal to maximize its nutritional value for poultry. *Anim. Feed Sci. Technol.* 47: 79-188. 132. Lee, G. G., Ferket, P. R., Shih, J. C. H. 1991. Improvement of feather digestibility by bacterial keratinase as a feed additive. *FASEB J.* 59: 1312. 133. Lee, H., Suh, D. B., Hwang, J. H., Suh, H. J. 2002. Characterization of a keratinolytic metalloprotease from *Bacillus* sp. SCB-3. *Appl. Biochem. Biotechnol.* 97: 123-133. 134. Lee, K. H., Park, K. K., Park, S. H. and Lee, J. B. 1987. Isolation, purification and characterization of keratinolytic proteinase from *Microsporum canis*. *Yonsei Med. J.* 28: 131-138. 135. Letourneau, F., Sousso, V., Bressollier, P., Branland, P. and Verneuil, B. 1998. Keratinolytic activity of *Streptomyces* sp. S. KI-02: a new isolated strain. *Lett. Appl. Microbiol.* 26: 77-80. 136. Li, E. and Yousten, A. A. 1975. Metalloprotease from *Bacillus thuringiensis*. *Appl. Microbiol.* 30: 354-361. 137. Lin, X., Inglis, G. D., Yanke, L. J. and Cheng, K. J. 1999. Selection and characterization of feather degrading bacteria from conola meal compost. *J. Ind. Microbiol. Biotechnol.* 23: 149-153. 138. Lin, X., Kelemen, D. W., Miller, E. S. and Shih, 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. 139. Lin, X., Lee, C., 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: 3271-3275. 140. Lin, X., Shih, J. C. H. and Swaisgood, E. H. 1996. Hydrolysis of feather keratin by immobilized keratinase. *Appl. Environ. Microbiol.* 62: 4273-4275. 141. Lindberg, R. A., Eirich, L. D., Price, J. S., Wolfenbarger, L. Jr. and Drucker, H. 1981. Alkaline protease from *Neurospora crassa* purification and partial characterization. *J. Biol. Chem.* 256: 811-814. 142. 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: 594-596. 143. Mallya, S. K., and Van Wart, H. E. 1989. Mechanism of inhibition of human neutrophil collagenase by gold (I) chrysotherapeutic compounds. Interaction at a heavy metal binding site. *J. Biol. Chem.* 264: 1594-1601. 144. 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. 145. Malviya, H. K., Rajak, R. C. and Hasija, S. K. 1993b. In vitro degradation of hair keratin by *Graphium penicillodeus*: evidences for sulfitolysis and peptidolysis. *Crypt. Bot.* 3: 197-201. 146. Malviya, H. K., Tiwari, S., Rajak, R. C. and Hasija, S. K. 1993a. Keratinolysis by four fungi isolated from the soil and effluent of a gelatin factory at Jabalpur (M.P.). *Crypt. Bot.* 3: 108-116. 147. Manczinger, L., Rozs, M., Vagvolgyi Cs and Kevei, F. 2003. Isolation and characterization of a new keratinolytic *Bacillus licheniformis* strain. *World J. Microbiol. Biotechnol.* 19: 35-39. 148. Matsubara, H. and Feder, J. 1971. *The Enzymes*. p. 721. Academic press, New York, USA. 149. Mayer, A. F. and Deckwer, W. D. 1996. Simultaneous production and decomposition of clavulanic acid during *Streptomyces clavuligerus* cultivations. *Appl. Microbiol. Biotechnol.* 45: 41-46. 150. Mercer, E. H. and Verma, B. S. 1963. Hair digested by *Trichophyton mentagrophytes*. An electron microscope examination. *Arch. Dermatol. (Chicago).* 87: 357-360. 151. Mignon, B., Swinnen, M., Bouchara, J. P., Holinger, M., Nikkels, A. and Pierard, G. 1998. Purification and characterization of a 315 kDa keratinolytic subtilisin like serine protease from *Microsporum canis* and evidence of its secretion in naturally infected cats. *Med Mycol.* 36: 395-404. 152. Mitsuiki, 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 *Nocardiopsis* sp. TOA-1. *Enzyme Microb. Technol.* 34: 482-489. 153. Moallaei, H., Zaini, F., Larcher, G., Beucher, B., Bouchara, J. 2006. Partial purification and characterization of a 37 kDa extracellular proteinase from *Trichophyton vanbreuseghemii*. *Mycopathologia.* 161: 369-375. 154. Mohamedin, A. H. 1999. Isolation, identification and some cultural conditions of a protease-producing thermophilic *Streptomyces* strain grown on chicken feather as a substrate. *Int. Biodeterior. Biodegradation.* 43: 13-21. 155. Mohammed El-Akied, Z. M. 1987. Microbial production of amino acids and proteins by thermophilic actinomycetes as a biodegradation of chicken feather. M. Sc. Thesis, Zagazig University, Egypt. 156. Mukhopadhyay, R. P. and Chandra, A. L. 1990. Keratinase of a *Streptomyces*. *Indian J. Exp. Biol.* 28: 575-577. 157. Moreira, F. G., de Souza, C. G. M., Costa, M. A. F., Reis S. and Peralta R. M. 2007. Degradation of keratinous materials by the plant pathogenic fungus *Myrothecium verrucaria*. *Mycopathologia.* 163: 153-160. 158. Morihara, K. 1974. Comparative specificity of microbial proteinases. *Adv. Enzymol. Relat. Areas Mol. Biol.* 41: 179-243. 159. Morihara, K. and Oda, K. 1992. Microbial degradation of proteins. In *Microbial degradation of natural products*, ed. G. Winkelmann. VCH Verlagsgesellschaft mbH, Weinheim, Germany. pp. 293-364. 160. Morihara, K., Tatsushi, O. and Tsuzuki, H. 1967. Multiple proteolytic enzymes of *Streptomyces fradiae*. Production, isolation, and preliminary characterization. *Biochim. Biophys. Acta.* 139: 382-397. 161. Mukhopadhyay, R. P. and Chandra, A. L. 1990. Keratinase of a *Streptomyces*. *Indian journal experimental biology* 28: 575-577. 162. Naguib, M. 1., Mohammed, N. K. and Yassin, A. F. 1984. Studies on Actinomycetes of Egyptian soils: Effect of continuous incubation on keratin hydrolysis with reference to peptide formation. *Egypt. J. Bot.* 22: 57-72. 163. Nakanishi, T. and Yamamoto, T. 1974. Action and specificity of a *Streptomyces* alkaliphilic proteinase. *Agric. Biol. Chem.* 35: 2391-2397. 164. Nam, G. W., Lee, D. W., Lee, H. S., Lee, N. J., Kim, B. C., Choe, E. A., Hwang, J. K., Suhartono, M. T. and Pyun, Y. R. 2002. Native-feather degradation by *Fervidobacterium islandicum* AW-1, a newly

isolated keratinase-producing thermophilic anaerobe. Arch. Microbiol. 178: 538-547. 164.Nilegaonkar, S. S., Zambare, V. P., Kanekar, P. P., Dhakephalkar, P. K. and Sarnail, S. S. 2007. Production and partial characterization of dehairing protease from *Bacillus cereus* MCM B-326. Bioresour. Technol. 98: 1238-1245. 165.Nishio, T. and Hayashi, R. 1984. Digestion of protein substrates by subtilisin: immobilization changes the pattern of the products. Arch. Biochem. Biophys. 229: 304-311. 166.North, M. J. 1982. Comparative biochemistry of the proteinases of eucaryotic microorganisms. Microbiol. Mol. Biol. Rev. 46: 308-340. 167.Noval, J. J., and Nickerson, W. J. 1959. Decomposition of native keratin by *Streptomyces fradiae*. J. Bacteriol. 77: 251-263. 168.Onifade, A. A., Al-Sane, N. A., Al-Musallam, A. A., Al-Zarban, S. 1998. Potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. Biores Technol. 66: 1-11. 169.Paisley, L. G. and Hostrup-Pedersen, J. 2005. A quantitative assessment of the BSE risk associated with fly ash and slag from the incineration of meat-and-bone meal in a gas-fired power plant in Denmark. Prev. Vet. Med. 68: 263-275. 170.Papadopoulos, M. C. 1985. Amino acid content and protein solubility of feather meal as affected by different processing conditions. Neth. J. Agric. Sci. (Netherlands) 33: 317-319. 171.Papadopoulos, M. C. 1986. The effect of enzymatic treatment on amino acid content and nitrogen characteristics of feather meal. Anim. Feed Sci. Technol. 16: 151-156. 172.Papadopoulos, M. C. 1989. Effect of processing on high protein feedstuffs: A review. Biol. Wastes. 29: 123-138. 173.Parry, D. A. D., and North, A. C. T. 1998. Hard -keratin intermediate filament chains: substructure of the N- and C-terminal domains and the predicted structure and function of the C-terminal domains of type I and type II chains. J. Struct. Biol. 122: 67-75. 174.Patel, B., K. and Jagannadham M. V. 2003. A high cysteine containing thiol proteinase from the latex of *Ervatamia heyneana*: purification and comparison with ervatamin B and C from *Ervatamia coronaria*. J. Sgric. Food Chem. 51: 6326-6334. 175.Patel, T. R., Jackman, D. M., Williams, G. J. and Bartlett, F. M. 1986. Extracellular heat resistant proteases of psychrotrophic pseudomonads. J. Food Prot. 49: 183-188 176.Peek, K., Daniel, R. M., Monk, C., Parker, L. and Coolbear, T. 1992. Purification and characterization of a thermostable proteinase isolated from *Thermus* sp. strain Rt41A. Eur. J. Biochem. 207: 1035-1044. 177.Pillai, P. and Archana, G. 2008. Hide depilation and feather disintegration studies with keratinolytic serine protease from a novel *Bacillus subtilis* isolate. Appl. Microbiol. Biotechnol. 78: 643-650. 178.Porro, A. M., Yoshioka, M. C. N., Kaminski, S. K., Palmeira, M. C. A., Fischman, O. and Alchorne, M. A. M. 1997. Disseminated dermatophytosis caused by *Microparium gypseum* in two patients infected with the acquired immune deficiency syndrome. Mycopathologia. 137: 9-12. 179.Porter, D. H., Swaisgood, H. E. and Catiagnani, G. L. 1984. Characterization of an immobilized digestive enzyme system for determination of protein digestibility. Agric Food Chem. 32: 334-339. 180.Rai