羽毛分解微生物之角蛋白

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摘要

從台灣彰化養雞場的羽毛廢棄物土壤中，篩選出3株具有分泌角蛋白酶能力之羽毛降解微生物。分離出來的Wu3、Wu4和Wu5菌株，經由微生物菌種鑑定後，顯示分别與Bacillus cereus strain QD232、Brevibacillus parabrevis M3和Bacillus thuringiensis strain INBI-2菌株的16S rDNA基因序列有99%相似。故將這3株分別命名為Bacillus cereus Wu3、Brevibacillus parabrevis Wu4和Bacillus thuringiensis Wu5。這3株羽毛降解微生物之菌株，是以羽毛作為碳、氮源，於37℃下培養4天，產生胞外角蛋白Q。胞外角蛋白Q在不同的pH和温度範圍下具有活性，且具有活性之溫度範圍分別為40-60℃、50-60℃和70-80℃。Bacillus cereus Wu3、Brevibacillus parabrevis Wu4和Bacillus thuringiensis Wu5角蛋白酶最適pH與溫度分別為pH 7、8和9與60、50和70℃。金屬螯合劑EDTA和O-phenanthroline蛋白酶抑制劑會對本研究角蛋白酶之酵素活性造成影響，因此這3種角蛋白酶皆與金屬蛋白酶有關。金屬螯合劑Ca2+、K+、Mg2+和Mn2+離子可增加酵素活性。另外，角蛋白酶在不同溫度和鹼性環境下，不同的輔因子會對酵素穩定性造成影響，根據研究結果顯示，添加Ca2+或Mn2+離子可增加酵素對熱的穩定性。Bacillus cereus Wu3和Brevibacillus parabrevis Wu4角蛋白酶以液態形式貯存於-20℃最具有穩定性，若以粉末形式存在於不同溫度下，酵素會快速失活。此外，添加一些有機溶劑和還原劑可增加酵素穩定性。特別是Bacillus cereus Wu3和Bacillus thuringiensis Wu5角蛋白酶對於不同角蛋白質基質具有寬廣水解能力，像是雞皮、鵝毛和豬毛角蛋白質基質。以偶氮酪蛋白為基質Wu3、Wu4和Wu5角蛋白酶之Km值，分別為2.31、0.98和0.95 g/L。

關鍵詞：角蛋白酶、羽毛廢棄物、金屬蛋白酶
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2.3 角蛋白

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參考文獻

1. 吳芝穎。2004。Bacillus licheniformis THSC-1 角蛋白分解酵素之純化、定性與基因選殖。東海大學畜產研究所碩士論文。台中，台灣。
2. 吳德盛、孫耀飛、郭憲峰。2005。毛粉的膨化加工。當代畜牧 7:41-43。高雄，台灣。
3. 沈潔瑩。2008。篩選角蛋白分解菌及其酵素性質研究。國立屏東科技大學食品科學系碩士論文。台東，台灣。
4. 林詩耀。2007。重油降解細菌之表現型及基因型特性研究。中興大學土壤環境科學系所碩士論文。台中，台灣。
5. 陳丹英、趙允、趙大中、翟中和。1998。鐵線蕨中間纖維的研究及某些植物類角蛋白的比較分析。植物學報 40(9):790-795。
6. 賈如琰、何玉鳳、王榮民、李芳蓉、王艷。2008。角蛋白的分子構成、提取及應用。化學通報 71(4):1-6。
alkalophilic bacteria grown on chicken feather. Enzyme microbial technology 32: 519–524.

Ghorbel, B., Sellami-Kamoun, A. and Nasri, M. Harcourt college pub, Belmont, USA.


for poultry. Animal feed science and technology 47: 179–188.


Latshaw, J.D., Musharaf, N. and Retrum, R. 1994. Processing of feather meal to maximize its nutritional value


–1789.

Larcher G., Cimon B., Symoens F., Tronchin G., Chabasse D. and Bouchara J. P. 1996. A 33 kDa serine proteinase from Scedoporium

Marcel dekker, New York, USA.


biochemistry 46: 331–358.


Kraut, J. 1977. Serine proteases: structure and mechanism of catalysis. Annual reviews of

–291.


94-96.


A. and Patterson, J. D. E. 1983. Partial purification of Mucor pusillus intracellular proteases. Applied and environmental microbiology 45:

thermostable alkaline protease from Bacillus subtilis NCIM No. 64. Applied biochemistry and biotechnology 38: 83–92.

Khan, M. R., Blain, J.

from Bacillus pumilus CBS with high catalytic efficiency. Biochimie 90: 1291-1305.


pennavorans bv. nov., a thermotolerant organism isolated from solfataric muds. Microbiological research 163: 105–112.


thermophilic actinomycete strain Thermoactinomyces candidus. Canadian journal of microbiology 45: 217–222.


Microbiology and biotechnology 59: 15–32.


Godfrey, T. and Reichelt, P. 1985. Industrial enzymology: the


