捌、英文摘要

Purification, Characterization and Cloning of the Keratinase from *Bacillus licheniformis* THSC-1

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Abstract

Our laboratory has reported the isolation of a keratinolytic bacterium, *Bacillus licheniformis* THSC-1 that effectively degrades feather waste and represents an alternative to improve the nutritional value of feather waste. The purpose of this study was to investigate the purification, characterization and cloning of the keratinase from *B. licheniformis* THSC-1, and to expect to have advantage of development of waste disposal and bioconversion in the livestock industry.

The maximum enzyme activity produced by *B. licheniformis* THSC-1 was incubated in feather culture medium at 50℃ for 4 days with shaking. The crude keratinase produced by the above-mentioned condition was purified 11.6-fold by ultrafiltration and cation exchange chromatography, and its activity was 4231.6 U/mg. This keratinase was demonstrated as an extracellular enzyme. Optimal activity was exhibited at 70℃ and pH 8.0. The enzyme was markedly inhibited by serine proteinase inhibitor–Phenylmethylsulfonyl fluoride（PMSF）. It was concluded that the

keratinase therefore belonged to the group of serine proteinases. The keratinase hydrolyzed a wide variety of protein substrates, including soluble casein and insoluble keratin such as feather, wool, pig hair and human hair. Further nucleotide sequence characterization of the keratinase demonstrated that the DNA fragment of 1137 bp was obtained. This keratinase gene shared a 99 % sequence identity with the gene encoding keratinase from *B. licheniformis* PWD-1and it showed that high homology with the subtilisin family of the keratinolytic *Bacillus* strains. This keratinase, therefore, belongs to the members of the subtilisin family.

玖、小傳

筆者吳芝穎係台灣省新竹市人，生於民國 68 年 6 月 26 日，先後畢業於台北市大安國小、弘道國中與大誠高中。民國 86 年考取私立東海大學畜產學系，於民國 90 年畢業，取得東海大學農學學士學位；並於同年考取母校畜產系研究所，追隨恩師 施宗雄教授研習畜產加工與應用微生物學。承蒙恩師於研究所求學期間之悉心指導與鼓勵，方可於民國 93 年 2 月順利完成此論文。

附錄一、*B. licheniformis* THSC-1 於食品工業發展研究所之鑑定報告

B. licheniformis THSC-1	
Morphology	Rod
Gram stain	＋
Oxidase test	－
Catalase test	＋
Motility	－
Glucose utilization	Fermentation
Anaerobic growth	＋
Spore	＋
Spore width ＞cell	＋
Growth nutrient BR	＋
Indole	－
Voges-proskauer	＋
Citrate（Koser’s）	＋
Growth 2 % NaCl	＋
Growth 7% NaCl	＋
Growth 45℃	＋
Growth 65℃	＋
發酵 D-Glucose 產酸	＋
發酵 D-Glucose 產氣	－
發酵 L-Arabinose 產酸	＋
發酵 D-Xylose 產酸	－
發酵 D-Mannitol 產酸	＋

（張，1999）

附錄二、緩衝溶液之組成與 pH 值

Buffer range(pH)	配方	用量（mL）	備註
3.0	1M HCl	48.5	定量至 250 mL
	1M 醋酸鈉	50	
4.0	1M HCl	40	
	1M 醋酸鈉	50	
5.0	1M HCl	15	
	1M 醋酸鈉	50	
6.0	0.2M NaOH	5.64	定量至 200 mL
	0.2M KH_2PO_4	50	
6.5	0.2M NaOH	20.67	
	0.2M KH_2PO_4	50	
7.0	0.2M NaOH	29.54	
	0.2M KH_2PO_4	50	
7.5	0.2M NaOH	37.12	
	0.2M KH_2PO_4	50	
8.0	0.05M $Na_2B_4O_7$	5.5	
	0.1M HCl	4.5	
8.5	0.05M $Na_2B_4O_7$	6.5	
	0.1M HCl	3.5	
9.0	0.05M $Na_2B_4O_7$	8.5	
	0.1M HCl	1.5	
9.5	0.05M $Na_2B_4O_7$	8.0	
	0.1M NaOH	2.0	
10.0	0.05M $Na_2B_4O_7$	6.0	
	0.1M NaOH	4.0	
11.0	0.05M $Na_2B_4O_7$	5.0	
	0.1M NaOH	5.0	

（賴與李，1976）

附錄三、蛋白酶抑制劑之製備

Inhibitor	Protease class	Stock solution	Working solution
E-64	Thiol protease	1mM	1-10 μM
Notes	Working solution is stable for days at neutral pH Stock solution is stable for months at -20℃		

Inhibitor	Protease class	Stock solution	Working solution
Pepstatin A	Acid protease	10mM	0.1-1 μM
Notes	It is souble in 100% EtOH or DMSO Working solution is stable for at least 1day Stock solution is stable for months at -20℃		

Inhibitor	Protease class	Stock solution	Working solution
PMSF	Serine protease	200mM	0.1-1 mM
Notes	It is souble in 100% EtOH or isopropanol The half-life of working solution at 25℃ pH 7.5 is 1 h Stock solution is stable for least 9 months at 4℃		

Inhibitor	Protease class	Stock solution	Working solution
EDTA	Metalloprotease	0.5M	1-10 mM
Notes	Stable for months at 4℃（pH8.5）		

（Beynon *et al*., 1989）