

Thesis Title	Purification and Characterization of Extracellular Protease Produced by Thermophilic Bacteria Strain TLS33	
Author	Mr. Supachok Sinchaikul	
M.S.	Biotechnology	
Examining Committee:	Associate Prof. Dr. Suree Phutrakul	Chairman
	Dr. Dararat Tongkao	Member
	Dr. Manee Pookanjanatavip	Member

Abstract

Thermophilic bacteria strain TLS33 producing extracellular thermostable protease was isolated from Thephanom hot spring in Chiang Mai. The bacteria grew and secreted high protease activity when cultured in a medium containing 0.1% w/v yeast extract and 0.25% w/v skim milk in 80% v/v base mixture at 65°C for 48 h. This protease exhibited the optimum pH and temperature for activity at 5.0-8.0 and 70 - 80°C, respectively. It was stable in a buffer solution pH 7.0. after incubated at 75°C for 1 h. Thermostabilities of the proteases in the presence and absence of thiol-reactive reagents were increased by the addition of CaCl₂. Diisopropyl ether, cyclohexane and hexane protected this protease from heat denaturation. The protease could hydrolyse a number of substrates including casein, lysozyme, skim milk, ovalbumin, hemoglobin, BSA, soybean, azocasein, creatinine and gelatin, respectively. It was completely inactivated by 40 mM EDTA which could be classified as a neutral metalloprotease. Furthermore, the proteolytic activity of the enzyme was enhanced by low concentration (< 10 mM) of Ca²⁺, Mg²⁺ and K⁺, especially the addition of 1mM Zn²⁺ activated the higher activity of the enzyme and restored the activity of the apoenzyme up to 64.94%. From these results, this protease could be classified as neutral metalloprotease containing Zn²⁺ in its molecule.

The protease was concentrated and partially purified by freeze-dry, dialysis, ion-exchange chromatography and gel filtration and its molecular weight determined by gel filtration was about 21,843 dalton. The characterizations of the partially purified protease was the same as the crude protease which exhibited the optimum pH and temperature for its activity at 5.0-8.0 and 70-80°C, respectively. It was stable up to 70°C and stable in 50% v/v organic solvents such as diisopropyl ether, cyclohexane and hexane. Its activity was almost completely lost in the presence of 5 mM EDTA and it was enhanced by low concentration (1 mM) of Ca²⁺, Mg²⁺ and K⁺, especially the addition of Zn²⁺. Thus, the metal ion in the partially purified protease molecule was Zn²⁺. The kinetic parameters of the partially purified protease (K_m and V_{max} values) when azocasein was used as substrate were 0.14 %w/v and 0.108 U/ml/min, respectively.