

Study on Characterization of Keratinases from Feather-Degrading Microorganisms

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ABSTRACT

Three feather-degrading microorganisms with keratinase producing capacity were isolated from poultry farm feather waste soil in Changhua. Sequence analysis of 16S rDNA sequence for the isolated Wu3, Wu4 and Wu5 strains microbial identification report showed 99% homology with *Bacillus cereus* strain QD232, *Brevibacillus parabrevis* M3 and *Bacillus thuringiensis* strain INBI-2, respectively. It was identified as *Bacillus cereus* Wu3, *Brevibacillus parabrevis* Wu4 and *Bacillus thuringiensis* Wu5, respectively. A extracellular keratinases produced by three strains grown on feather as carbon and nitrogen source after liquid culture at 37 °C for 4 days. Three keratinases (*Bacillus cereus* Wu3, *Brevibacillus parabrevis* Wu4 and *Bacillus thuringiensis* Wu5) exhibited activity at pH range of pH 6.5-8.0, pH 7.0-8.5 and pH 7.5-11.0, temperature range of 40-60 °C, 50-60 °C and 70-80 °C, respectively, with azo-casein as substrate. Optimum pH and temperature of *Bacillus cereus* Wu3, *Brevibacillus parabrevis* Wu4 and *Bacillus thuringiensis* Wu5 keratinases were pH7, pH8 and pH9 and 60 °C, 50 °C and 70 °C, respectively. The protinase inhibitory effect of metal chelator EDTA and O-phenanthroline characterized three keratinases as metalloproteases. The three bacterial keratinases were completely activated by the presence of Ca²⁺, K⁺, Mg²⁺ and Mn²⁺. In addition, characterization of three keratinases and the effect of various co-factors on the stability at various temperature and in alkaline pH range was carried out. The results of the present investigation showed that the enzyme was fairly stable to heat treatment in exist of Ca²⁺ or Mn²⁺. *Bacillus cereus* Wu3 and *Brevibacillus parabrevis* Wu4 keratinases were stable as liquid storage at -20 °C, nevertheless the keratinases activity started to drop significantly as powder storage at difference temperature. Further, the three enzymes showed enhance stability in the presence of some organic solvents and reducing agents. The keratinases hydrolyzed a wide of keratin substrates, including chicken skin, goose feather and pig hair, especially *Bacillus cereus* Wu3 and *Bacillus thuringiensis* Wu5 keratinases. The Km of *Bacillus cereus* Wu3, *Brevibacillus parabrevis* Wu4 and *Bacillus thuringiensis* Wu5 keratinases with azo-casein as substrate were 2.31, 0.98 and 0.95 g/L, respectively.

Keywords : keratinase、 feather waste、 metalloproteases

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