Isolation of Keratinase-Producing Microorganisms, Analysis of Enzyme Characteristics and Applications

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ABSTRACT

Six feather-degrading microorganisms with keratinase producing capacity were isolated from poultry farm feather waste soil in Changhua. They were identified by sequence analysis of 16S rDNA, and named as Bacillus megaterium Wu1, Bacillus cereus Wu2, Bacillus cereus Wu3, Brevibacillus parabrevis Wu4, Bacillus thuringiensis Wu5 and Bacillus cereus Wu6, respectively. The optimum initial pH value of medium to Wu1, Wu2, Wu4, Wu5 and Wu6 was pH 5.0, but Wu3 was pH 9.0. The optimum culture temperature to these six strains between 30 and 40oC. The extracellular keratinases produced by six strains grown on feather as carbon and nitrogen source after liquid culture with each optimum cultured conditions for 4 days. Four keratinases (B. megaterium Wu1, Bacillus cereus Wu3 and Bacillus thuringiensis Wu5 and Bacillus cereus Wu6) were purified by ammonium sulfate precipitation, Sephacryl S-200 HR gel filtration column and DEAE Sephadex A-50 ions exchange column. By these steps, the purity of these enzymes increased by 7.63, 19.48, 2.23 and 4.71 fold, with activity recovery of 13.59%, 26.32%, 16.60% and 10.55%, respectively. The molecular mass of these enzymes determined by SDS-PAGE was 34, 46, 32, 55, 68 kDa, respectively, the keratinase Wu6 was a dimeric protein. These purified enzymes exhibited activity at pH range of pH 4.0-12.0 and pH 6.0-11.0 temperature range of 10-100oC, respectively, with azo-casein as substrate. Optimum pH and temperature of B. megaterium Wu1 and B. cereus Wu6 keratinases were pH 7, and pH8, and 50oC, 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 Na+ and Mg2+. B. megaterium Wu1 and B. cereus Wu6 keratinases were stable as powder storage at various temperature, nevertheless the keratinases activity started to drop significantly as liquid storage at room temperature. Further, the keratinase Wu1 showed enhence stability in the presence of some organic solvents, but reducings agents were inhibited the keratinase activity from B. megaterium Wu1 and B. cereus Wu6. The Km of B. megaterium Wu1 and B. cereus Wu6 keratinases with azo-casein as substrate were 0.85 and 3.28 g/L, respectively.

Keywords : keratinase、feather waste、metalloproteases

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