

Purification and Characterization of Chitinase and Protease from a Bacteria Strain TKU008

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ABSTRACT

The purpose of this study is to isolate an indigenous microorganism to degrade Shrimp and Crab shell Powder (SCSP) chitin and to secrete extracellular protease and chitinase. To found the optimal condition for protease and chitinase production. Extracellular proteins were purified by ammonium sulfate precipitation, dialysis to remove salts, ionic exchange of DEAE-sepharose CL-6B and sephadex S-100 gel filtration. The molecular mass of TKU008 protease and chitinase was determined by SDS-PAGE and gel filtration was 40 kDa and 57 kDa, approximately. Bacterium TKU 008 was isolated from soil of southern of Taiwan. Shrimp and Crab shell Powder was used to be the carbon source of TKU008. The optimized culture condition for protease and chitinase production was found when culture was shaken in 100 mL of medium (pH6) at 30 °C containing: 1% SCSP, 0.1% K₂HPO₄, 0.05% MgSO₄ · 7H₂O. The highest yield of protease and chitinase was produced under the optimum culture condition. The optimum pH, optimum temperature, pH stability and thermal stability of protease were pH 7, 50 °C, pH 6 and 30 °C. The optimum pH, optimum temperature, pH stability and thermal stability of chitinase were pH 6, 50 °C, pH 7 and 30~40 °C. After the purification, the yield, fold and specific activity of protease are 1%, 2-fold and 0.36 U/mL and the yield, fold and specific activity of chitinase are 50%, 134-fold and 7.56 U/mL by purification procedures. The protease was characterized as a metalloprotease, due to it was inactivated by EDTA. Both of protease and chitinase activities were inhibited by Cu²⁺、Mn²⁺. When enzymes were treated of various surfactants, either protease or chitinase activity were stable. The effect of organic solvents on protease and chitinase activity were also explored. The remaining activity of TKU008 protease was 90% after keeping it at 25 °C and 4 °C for 10 days. Enzyme had different specific proteolytic activity with casein、elastin、human albumin、hemoglobin as the substrates.

Keywords: chitin; protease; chitinase; shrimp and crab shall power

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