The study of protease produced by Pseudomonas aeruginosa K-187

歐宜書、王三郎

E-mail: 8701179@mail.dyu.edu.tw

ABSTRACT

The fermentation broth of the chitinolytic bacterium Pseudonomas aeruginosa K-187, isolated from soil in the northern Taiwan, was found to not only posses chitinase and lysozyme activities, but also posses protease activity. Under the optimized culture condition that the culture was shaken in 250 mL erlenmeyer flasks with 50 mL medium （5%shrimp and crab shell powder（SCSP）,0.1%K2HPO4, 0.5%MgSO4,1.0%lactose, 0.5%NH4NO3, and 0.5%FeSO4・7H2O, pH 8.0adjusted by phosphate buffer solution）at 25°Cfor 2 days, the proteaseactivity of P. aeruginosa K-187 was as high as 21.2U/mL . It was about ten fold of the activity （2.20U/mL）of P. aruginosa K-187 under the unoptimized basal culture condition. The protease of P. aeruginosa K-187 in the optimum enzyme condition was used to test as protein cleaner. In liquid state culture, the efficiency of protein cleaning of SCSP was 72%, while that of natural shrimp shell（NSS）was up to 78%. In the unoptimized condition, the efficiencies of protein cleaning for SCSP and NSS were 48%and 60%, respectively. In comparsion with those of Eurotium repens, Monascus purpureus and Bucillus subtilis, the efficiency of protein cleaning of SCSP for P. aeruginosa K-187 a the highest one. The protease of P. aeruginosa K-187, produced under the optimized culture condition, first was precipitated and dialyzed by using ammonium sulfate. The further purification and separation procedures of the protease were processed by the use of DEAE-Sepharose CL-6B ionic exchange chromatography and Sephacryl S-200 gel permeation chromatography. Purification was 19-fold with the crude enzyme solution. After purification and separation the activity of the protease enzyme was still stable at 50°Cand pH 7?9, while the optimal temperature and pH for the enzyme reaction were at 50°Cand pH 8, respectively. By SDS-PAGE electrophoresis, the molecular weight of the protease was identified 58.8KDa. The protease produced from P. aeruginosa K-187 can covalently linked to the supporting AS-L（hydroxypropyl methyl cellulose acetate succinate）polymers.!Therefore, the crude solution of protease was used for enzyme immobilization with the AS-L polymer carriers. The efficiency of enzyme immobilization was 82%. The pH- and heat-stability ranges of the immobilized enzyme were at pH 6?9 and 60°C, respectively . The optimal enzyme reaction for the immobilized protease activity were under 50°Cand pH 8.0. The efficiency of SCSP protein cleaning by using the immobilized protease was only 67%and a little lower than that by using the crude protease solution.

Keywords : 去蛋白 ; 蛋白質分解脢 ; 固定化

Table of Contents