

**P35 Characterization of Partially Purified Extracellular  
Protease of Local Bacteria BAC-4**

**Siswati Setiasih**

Dept. of Chemistry, Faculty of Mathematics and Science  
University of Indonesia, Indonesia

To achieve the aim of this investigation, the extracellular protease was isolated from bacteria BAC-4, a strain was cultivated in the medium for the production of penicillin acilase in a period of 32 hours.

The enzyme was first purified by acetone precipitation method, followed by ion exchange chromatography on DEAE-sephacel column. The highest specific activity of the acetone fraction was found to be 2.19 unit per mg, with degree of purification of 13 times. Further purification of the enzyme on DEAE-sephacel had a specific activity of 58.6 unit per mg and degree of purification of 344 times compared to its crude extract.

The optimum pH of the enzyme was 8.4, and the optimum temperature was 37 °C. The  $K_M$  and  $V_{max}$  calculated at experiment conditions were found to be 0.66%(W/V) and 3.61 unit per mL respectively.

Its proteolytic activity of the enzyme was inhibited by EDTA and PCMB, but it was activated by calcium chloride and cysteine.

In general, it could be concluded that the protease was a metalloenzyme.