Chapter 4

Conclusion

1. Effect of cultural medium and environmetal conditions on growth and fibrinolytic enzyme production

S. commune BL 23 was cultivated under submerged cultivation for the production of fibrinolytic enzyme. The culural medium, initial pH of the culture medium, incubation period, cultivation temperature and shaking speed were investigated.

The maximum growth and fibrinolytic enzyme production were achieved when *S. commune* BL 23 was cultured in peptone yeast extract glucose medium (PYGM) with the initial pH of 6.0 at 35°C, 150 rpm for 7 days. The maximum biomass and the fibrinolytic enzyme activity were 8.93 g/l and 576.73 U, respectively.

2. Partially purified fibrinolytic enzyme

2.1 The protein from cultural supernatant of *S. commune* was concentrated by fractional precipitation with 60-80% saturation of ammonium sulfate, dialysed with 20 mM Tris-HCl, pH 7.0 and further purified using anion exchange chromatography (DEAE-Sephacel column). Specific activity of 39.31×10^4 units/mg protein were obtained. The purification factor was increased about 86 fold with the yields of 36.47%.

2.2 The protein band from native polyacrylamide gel electrophoresis showed the activity of fibrinolytic enzyme on the fibrin plate.

3. Characterization of partially purified fibrinolytic enzyme

3.1 Optimal temperature for fibrinolytic enzyme activity was 50°C.

3.2 Fibrinolytic enzyme was stable in the pH range of 5.0-11.0 with the residual activity over 70%. The enzyme was stable at the temperature range of 40-50°C but the activity was totally lost at 60°C for 48 h. Moreover, it was stable at 30°C for 60 days.

3.3 Fibrinolytic activity was inhibited by 1,10-phenanthroline and EDTA and its activity was gradually decreased when the concentration of EDTA was increased. Hg^{2+} totally inhibited the enzyme activity. However, its activity was not inhibited by PMSF and SBTI. It is indicated that the enzyme is a metalloprotease.