

Properties of Thermostable Proteases with Fibrinolytic Activity from Local Earthworm Extracts (*Lumbricus rubellus* and *Pheretima asiatica*)

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Earthworm extract was widely used for traditional medicine in oriental countries for centuries. Recently, earthworm extract were studied further for its potential application for treatment of thrombosis diseases. Earthworm could secrete a group of proteases with fibrinolytic activity which known as lumbricinase. In this research, we explored the resistance of enzymes toward high temperatures and characterized its properties. Two local earthworms (*Lumbricus rubellus* and *Pheretima asiatica*) were extracted in phosphate buffer (pH 7) to produce crude enzymes. Each crude was heated at range of high temperatures (50-80°C) for about 5-20 min. Activity of protease and protein content were analysed. Fibrinolytic activity was detected by using zymogram analysis with fibrin copolymerization.

Both earthworm extracts showed optimal activity after heating treatment at 65°C for 10 min with specific activity 3,526 U/mg (for *L. rubellus* extract) and 3,872 U/mg (for *P. asiatica* extract). The optimal temperature and pH of thermostable enzymes were reached at 65°C and pH 9 (universal buffer) respectively. These enzymes were totally inhibited by 1 mM PMSF (phenylmethylsulfonyl fluoride), a specific serine protease inhibitor. Addition of 0,01 mg/mL STI (soybean trypsin inhibitor) sharply decreased a half enzyme activity from *L. rubellus* extract. Meanwhile, *P. asiatica* enzyme extract was also strongly inhibited by 0,1 mM TLCK (N α -p-tosyl-L-lisin klorometilketon) with residual activity 12%. Casein and fibrin zymogram analysis resulted in the specificity of thermostable enzyme to degrade both protein substrates well.

Keywords : fibrinolytic activity, *Lumbricus rubellus*, *Pheretima asiatica*, thermostable enzymes