Partial Purification of a Thermostable Lipase From a Local Strain of Geobacillus stearothe.rmophilus and Its Zymographic Analysis

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Background & Objectives: Lipases or triacylglycerol acylhydrolases (E.C.3.1.1.3) are enzymes that catalyze both the hydrolysis of triglycerides in lipid-water interface, and reverse reaction, ester synthesis in low-water systems. Lipases, specially thermostable ones, have wide applications in food and pharmaceutical industries, organic synthesis, and detergent formulation and have emerged as the third important industrial enzymes in biotechnology.The aim of this study is extraction and partial purification of an extracellular lipase from a local strain of *Geobacillus stearothermophilus* by ammonium sulfate precipitation Methods, optimization of salt concentration and also detection of active purified lipase using zymogram.

Methods: Ammonium sulfate precipitation (20-90% saturation) and dialysis were used for enzyme extraction and partial purification and desalting, respectively. Precipitated proteins were electrophoresed by sodium dodecyl sulfate polyacrylamide discontinuous gel electrophoresis (12% SDS-PAGE). To perform zymogram and determination of enzyme molecular weight, non-reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis was used. After electrophoresis, the gel was submerged in renaturation buffer containing Triton X-100, transferred on solid medium containing tributyrin and incubated at 60ºC.

Results: The results show that most of the enzyme precipitates in 70% saturated ammonium sulfate, leading to18.02-fold purification with a specific activity of 70.7 U/mg protein and recovery of 4.84%.

Conclusion: Zymographic analysis and observation of a clear band due to tributyrin hydrolysis demonstrate the purified enzyme has lipase activity even after heat treatment at 80 ºC. Its molecular weight was determined to be 61.92 kDa based on non-reducing SDS-PAGE.

Keywords: Lipase; *Geobacillus stearothermophilus*; Purification; Zymogram