

Secretory expression and characterization of a highly Ca²⁺-activated thermostable L2 lipase

ABSTRACT

Thermostable lipases are important biocatalysts, showing many interesting properties with industrial applications. Previously, a thermophilic *Bacillus* sp. strain L2 that produces a thermostable lipase was isolated. In this study, the gene encoding for mature thermostable L2 lipase was cloned into a *Pichia pastoris* expression vector. Under the control of the methanol-inducible alcohol oxidase (AOX) promoter, the recombinant L2 lipase was secreted into the culture medium driven by the *Saccharomyces cerevisiae* α -factor signal sequence. After optimization the maximum recombinant lipase activity achieved in shake flasks was 125 U/ml. The recombinant 44.5 kDa L2 lipase was purified 1.8-fold using affinity chromatography with 63.2% yield and a specific activity of 458.1 U/mg. Its activity was maximal at 70 °C and pH 8.0. Lipase activity increased 5-fold in the presence of Ca²⁺. L2 lipase showed a preference for medium to long chain triacylglycerols (C10-C16), corn oil, olive oil, soybean oil, and palm oil. Stabilization at high temperature and alkaline pH as well as its broad substrate specificity offer great potential for application in various industries that require high temperature operations.

Keyword: Recombinant thermostable lipase; *Pichia pastoris*; Ca²⁺ activation; High yield purification; Recombinant lipase; Thermostable