Catalytic Profiling of Partially Purified Bacterial Lipase

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ABSTRACT

Introduction: Microbial triacylglycerol hydrolases (EC 3.1.1.3) constitute a highly valuable class of biocatalysts due to their broad substrate versatility and the feasibility of large-scale production. In addition to their central role in lipid hydrolysis, these enzymes are also capable of catalyzing esterification, transesterification, and interesterification reactions. Among microbial sources, bacterial triacylglycerol hydrolases exhibit remarkable diversity in catalytic behavior and substrate specificity. Notably, enzymes derived from Pseudomonas species demonstrate exceptional tolerance to organic solvents, making them particularly suitable for a wide range of industrial bioprocesses. The major sectors utilizing these enzymes include the food, pharmaceutical, and chemical industries, where triacylglycerol hydrolases are routinely employed for various synthetic and degradative reactions. For industrial applications, highly homogeneous enzyme preparations are generally not required, except in sectors such as pharmaceuticals, cosmetics, and fine chemicals, where higher purity is essential. Nevertheless, purification is necessary when determining the primary structure of a triacylglycerol hydrolase protein. Additionally, purification steps are often implemented to enhance the enzyme's catalytic efficiency, operational stability, and shelf life.

Objectives: The present research focused on the partial purification of lipase produced by a newly identified *Bacillus* strain. The isolated enzyme was characterized with respect to its biochemical properties. The stability profiling was also assessed under varying physicochemical conditions of various detergents and metals.

Methodology: The bacterial isolate *Bacillus subtilis* SS4 was used for the production of triacylglycerol hydrolase at 40 °C in an oil-enriched medium (pH 7.0) with a fermentation period of 48 hours. The production medium was supplemented with palm oil (10.0 g/L), yeast extract (40.0 g/L), KH_2PO_4 (0.1 g/L), and 2% Ca^{2+} ions. Purification of triacylglycerol hydrolase initiated with biomass removal by centrifugation at 1,000 × g and 4 °C. Cell free supernatant was saturated with (10%) ammonium sulphate with continuous stirring at 4°C and kept overnight at 4°C followed by centrifugation at 10,000 rpm for 10 minutes. The pellets obtained were dissolved in minimum volume of 50mM phosphate buffer; pH 7.0 Then enzyme activity and protein content of cell free filtrate and Ammonium sulphate fraction was determined. The same procedure was repeated. After third run Ammonium sulphate fraction was dialyzed against 50mM Phosphte buffer (pH 7.0) for 24 hours at 4°C in a Dialysis bag. The concentrated enzyme after dialysis was obtained, Enzyme fractions were collected and the protein content was measured spectrophotometrically at 410 nm.

The partially purified triacylglycerol hydrolase was further examined for its catalytic profile.

Key Findings: The enzyme exhibited maximum activity at pH 6.0 and 40 °C, indicating its preference for mildly acidic conditions. Lipase activity increased with temperature up to 40 °C, after which a sharp decline was observed, with the highest activity recorded as 58.409 µmol/ml/min at 40 °C and the lowest at 80 °C. pH stability analysis showed that the enzyme retained more than 40% residual activity between pH 5.0 and 7.0, while significant deactivation occurred at extreme acidic and alkaline pH values.

The stability profile was further evaluated in the presence of detergents, surfactants, and metal ions. Tween-80 and Triton X-100 exerted minimal inhibitory effects, whereas SDS, EDTA, β-mercaptoethanol, and DMSO significantly reduced enzymatic activity, indicating sensitivity to denaturing agents and chelators. Conversely, metal ions such as Cu^{2+}, Fe^{3+}, Cd^{2+}, Mn^{2+}, Ag^{+}, Ca^{2+}, and Mg^{2+} enhanced lipase activity, suggesting that the enzyme may function as a metalloprotein.

Conclusion: In conclusion, the results demonstrate that the lipase from this *Bacillus* strain exhibits favorable activity and stability for potential industrial applications, particularly under mildly acidic and moderate-temperature conditions. Its metal-ion responsiveness and compatibility with nonionic surfactants further highlight its suitability as a robust biocatalyst for food, detergent, and bioprocess industries.

Keywords: *Bacillus Subtilis*, Triacylglycerol hydrolase, characterization, stability profile.

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