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Characterization of a thermostable-organic solvent-tolerant lipase from thermotolerant *Rhizopus* sp. strain PKC12B2 isolated from palm kernel cake

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Abstract

In this work, a new thermostable and organic solvent-tolerant lipase produced from *Rhizopus* sp. strain PKC12B2 was characterized to evaluate its activity and stability toward pH, temperature, organic solvents, and surfactants. The catalytic efficiency of the produced enzyme was also analyzed through enzyme kinetics study. Produced lipase exhibits remarkable stability at pH 8.0 to pH 10.0 and shows optimum activity at pH 9.0, demonstrating alkali lipase properties. Lipase has an optimum temperature of 45 °C and possesses significant thermal stability in the temperature range between 45 and 55 °C. Exceptional enzyme stability was also demonstrated in the presence of both polar solvents, such as glycerol, isopropanol and acetone, as well as non-polar solvents, such as cyclohexane and heptane up to 30 % (v/v) solvent concentration, where the enzyme retained more than 80 % activity after 1-h pre-incubation. For methanol, ethanol, and n-octanol, the relative activity of lipase was highly stable in the presence of up to 20 % (v/v) solvent concentration. Na⁺, Zn²⁺, and Fe³⁺ ions significantly stimulate the lipase activity at a final concentration of 10 mM. Lipase also demonstrated stimulatory effects towards a broad concentration range (0.2–1 %) of surfactants Tween 80 and Triton X-100. The Linearized Michaelis-Menten model in the form of Lineweaver-Burk (R² = 0.995) and Hanes-Woolf (R² = 0.996) models was found to successfully display the enzyme kinetics, in which the former generated Km and Vmax of 1.965 mM and 4.51 mM/min respectively, while the latter generated Km and Vmax of 1.295 mM and 3.389 mM/min, respectively. These new enzyme attributes demonstrate its great potential in biotechnology applications, such as for detergent formulations, biodiesel production and organic synthesis industries. © 2024 The Authors

Author Keywords

Characterization; Lipase; *Rhizopus* sp. strain PKC12B2; Thermostable-organic solvent-tolerant enzyme

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