

# Lipolytic enzymes of *Geobacillus* sp. 95: potential for industrial application



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## BACKGROUND

*Geobacillus* bacteria show high potential as biocatalysts suitable for industrial biotechnology applications. The ability of these bacteria to produce a variety of extracellular enzymes, such as amylases, xylanases, proteases, lipases, esterases and ureases has ranked them among the most important enzyme producers [1; 2]. Lipases are serine hydrolases that catalyse a wide range of bioconversion reactions: hydrolysis, transesterification and/or ester synthesis. Esters derived from these reactions are used in biofuel production or included in composition of cosmetics/perfumery. Thermostable and thermoactive lipolytic enzymes have gained remarkable importance over other industrially used biocatalysts due to their versatility regarding catalytic behavior. The main advantages of performing industrial processes at higher temperatures are reduced risk of microbial contamination and lower viscosity [1]. Therefore, it is important to characterize novel lipases/esterases and investigate the possibility to perform ester synthesis using such novel enzymes.

## OBJECTIVE

The main goal of this research was to investigate the potential of recombinant variant of GD-95 lipase and GDEst-95 esterase from *Geobacillus* sp. 95 for the synthesis of mono- and diacylglycerols and esters.

## MATERIALS AND METHODS

In this work lipase/esterase activity was measured spectrophotometrically using *p*-NP dodecanoate as substrate [3]. Activity of target enzymes at different temperatures was evaluated by carrying out the lipase assay at temperatures ranging from 5 to 90 °C. The effect of temperature on GD-95 lipase and GDEst-95 esterase stability was investigated by measuring the residual activity at 55 °C after incubation for 30 min at 30-90 °C. The formation of esters and mono- and diacylglycerols in the esterification or hydrolysis reaction mixtures was analyzed by thin-layer chromatography on boric acid-impregnated silica gel plates. The mobile phase mixture was composed of petroleum ether, diethyl ether, and glacial acetic acid (85:15:2 v/v/v). The chromatograms were developed with iodine vapour.

Our work shows that *Geobacillus* sp. strain 95 produces two types of thermostable, thermoactive and organic solvent-tolerant lipolytic enzymes: a lipase (named GD-95) and a carboxylesterase (named GDEst-95) [3-5]. Both enzymes displayed an ability to perform catalysis at temperatures ranging from 5 °C to 75 °C (Fig. 1a) while retaining more than 50 % of lipolytic activity after incubation at temperature range of 30-65 °C (Fig. 1b).

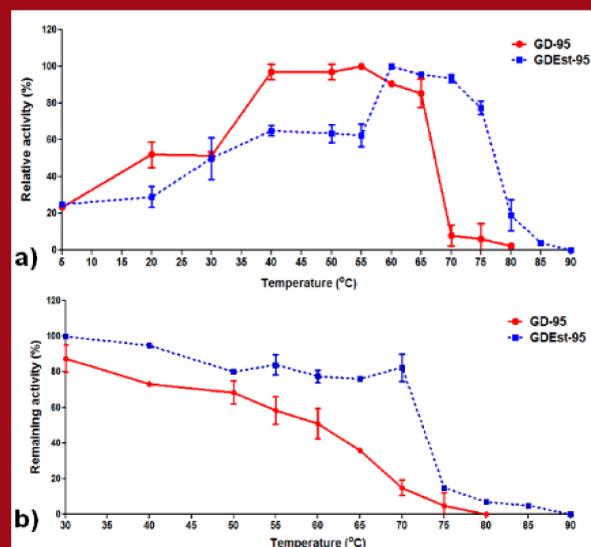


Fig. 1. The temperature effect on the lipolytic activity (a) and stability (b) of recombinant GD-95 lipase and GDEst-95 esterase [3; 5].

## CONCLUSIONS

The present study demonstrated that lipolytic enzymes produced by *Geobacillus* sp. strain 95 have suitable properties for industrial applications (thermoactivity, thermostability and tolerance to organic solvents). GD-95 lipase also can offer an eco-friendly alternative for chemical synthesis of esters or mono- and diacylglycerols via esterification of natural oils.

## RESULTS

Both biocatalysts also possess long-term (216 h) stability in isopropanol, methanol and hexane (25 % and 50 %) (Fig. 2a and 2b).

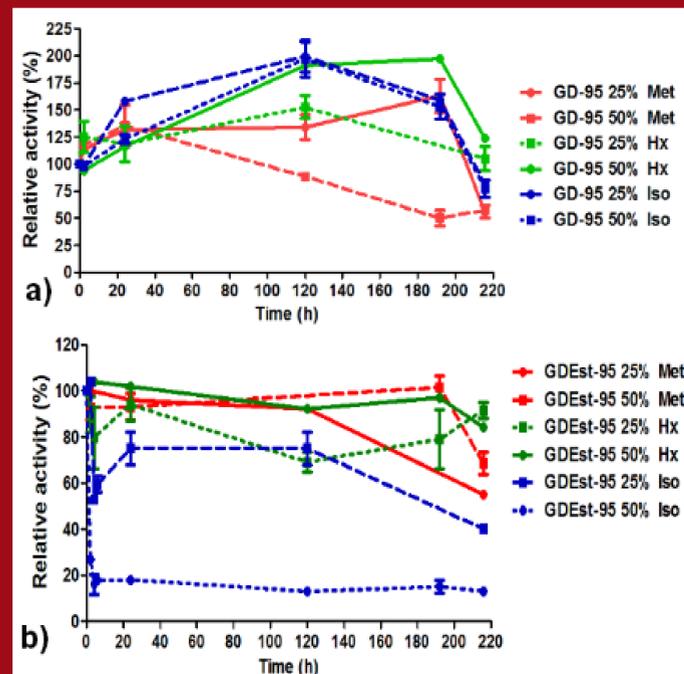


Fig. 2. The effect of organic solvents on lipolytic activity of GD-95 lipase (a) and GDEst-95 esterase (b). Met – methanol; Hx – hexane; Iso – isopropanol.

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Because of the stability of GD-95 lipase in organic solvents, the ability of this enzyme to perform ester synthesis was analyzed. Different natural oils and methanol or ethanol were used as substrates in this eco-friendly esterification reaction. Our results showed that new esters can be obtained using a mixture composed of coconut, peach, macadamia or other oils, 1/10 (w/w) ethanol or methanol and GD-95 lipase as biocatalyst. Results also suggested that GD-95 lipase can be a powerful tool for the production of emulsifiers (mono- or diacylglycerols) (Fig. 3).

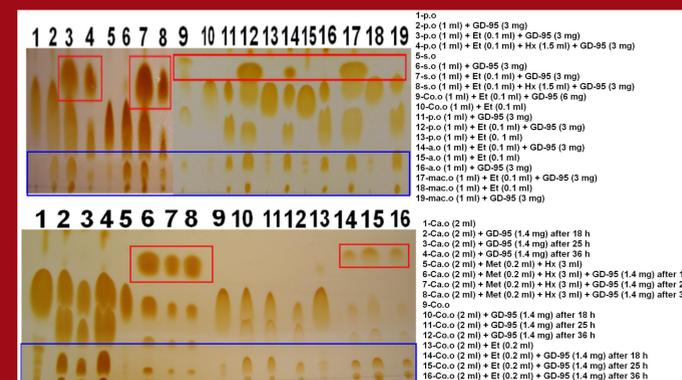


Fig. 3. Thin layer analysis of products obtained via esterification of peach, canola, coconut, macadamia and other oils with ethanol or methanol, using GD-95 lipase as biocatalyst. The esterification or hydrolysis reactions were performed at 25 °C, 18 h (unless stated otherwise). Red rectangles indicate esters; blue – mono- and diacylglycerols; Ca.o – canola oil; Co.o – Coconut oil; Et – ethanol; Hx – hexane; Met – methanol; p.o – peach oil; s.o – sunflower oil; a.o – argan oil; mac.o – macadamia oil.

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