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Biochemical analysis of *Staphylococcus saprophyticus* GTC1 natively secreted lipase

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BACKGROUND

Today microbial lipases find vast applications and have firmly established themselves in diverse areas of industrial microbiology and biotechnology. Among hydrolytic enzymes which are currently published to be the most widely used in industrial processes, lipases represent the third most commercialized group of enzymes and their production need is constantly increasing. For that reason, there is a perpetual interest in obtainment of low-cost, highly active and stable lipases which could be applied in different biotechnology branches. Furthermore, from the industrial point of view, usually, immobilized enzymes are preferred over their free forms since immobilization not only improves enzyme features but also makes it possible to reuse the enzyme and increases its turnover. Whereas easily cultivable bacteria are one of the cheapest sources of lipases with different suitable features, in the present study, potential of *Staphylococcus saprophyticus* as an enzyme producer was assayed.

AIMS OF THE WORK

•Purification of the secreted by *S. saprophyticus* LipGTC lipase;
•Determination of the main biochemical properties of the enzyme (optimal temperature, pH, thermostability, effect of various detergents, solvents and metal ions on the activity of the enzyme);
•Adsorption-immobilization of the enzyme and evaluation of the activity of its immobilized form;

MATERIALS AND METHODS

LipGTC was precipitated with ammonium sulphate from the medium (TSB) where it was secreted. Partially purified enzyme was analysed using SDS-PAGE and zymography. For the further analysis enzyme was eluted from the electrophoresis gel. LipGTC activity was measured spectrophotemtrically using *p*-NP butyrate as a substrate. Activity of LipGTC at different temperatures and pH was determined by measuring activity at temperatures ranging from 5 to 75°C and pH from 3 to 9, respectively. Thermostability of LipGTC was studied by measuring the residual activity at 35°C after incubation for 30 min at 20-80°C. Substrate speifity was measured with different *p*-NP (*p*-NP acetate, butyrate, octanoate, decanoate, dodecanoate, myristate, palmitate, stearate) substrates at 35ºC, pH 9. Effect of detergents (Tween 40, 60, 80, urea, SDS), organic solvents (methanol, n-hexane, DMSO, acetone) and various metal ions (K^+, Li^+, Mn^{2+}, Mg^{2+}, Ni^{2+}, Ba^{2+}, Ca^{2+}, Zn^{2+}, Fe^{3+}) on the activity of LipGTC was determined by measuring residual enzyme activity at 35ºC after 30 min of incubation with each chemical substance. The ability of LipGTC to hydrolyze natural fatty substrates was analyzed by thin-layer chromatography. The chromatograms were visualized in iodine vapour chambers. For hyperactivation studies of LipGTC , the enzyme was adsorption-immobilized on octyl-sepharose.

RESULTS

Secretion of the enzyme (LipGTC) was obtained in TSB liquid medium without addition of any inductors. After $(NH_4)_2SO_4$ precipitation, using SDS-PAGE and tributyrin overlay zymography, LipGTC was determined to have molecular mass of ~50 kDa (Fig. 1). LipGTC was eluted from the gel and its properties were further analyzed.

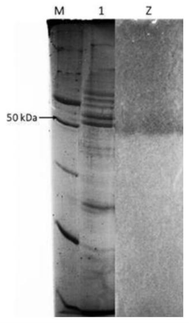


Fig. 1. *SDS-PAGE (on the left) and zymogram of partly purified LipGTC1 lipase. 1 - protein profile. Z - zymogram with visible clear zones of the tributyrin corresponding to protein band on the SDS-PAGE at the level of 50 kDa molecular mass marker (PageRuler™ Unstained Broad Range Protein Ladder).*

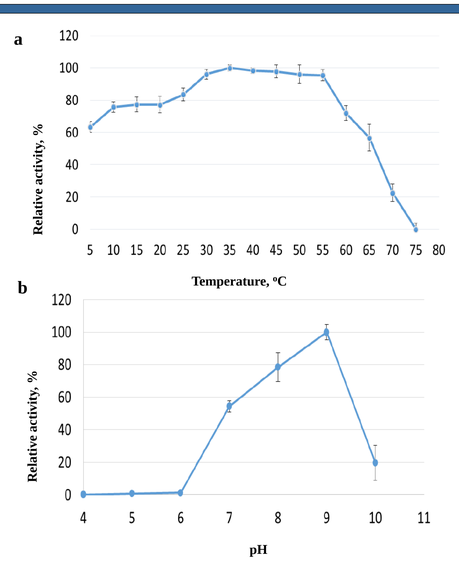


Fig. 2. *Effect of temperature (a) and pH (b) on LipGTC activity.*

Enzyme had optimal activity at 35 – 50ºC, pH 9 (Fig. 2 a, b) and hydrolyzed synthetic *p*-NP substrates with highest activity towards *p*-NPP (Fig. 3, a). LipGTC showed to be highly stable in the range of 30 – 50ºC up to 6 h without remarkable loss of activity (Fig. 3, b)

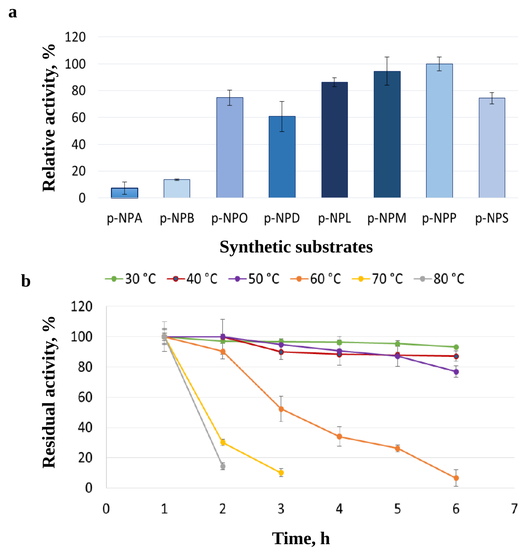


Fig. 3. *Substrate specifity (a) and thermal stability of LipGTC.*

LipGTC was also able to hydrolyze different natural lipids (olive, flaxseed, pumpkin seeds, rapeseed, sunflower and olive oils) releasing highly valuable fatty acids (Fig. 4 a, b). Some tested chemicals (detergents, different metal ions), as well as immobilization of the enzyme onto octyl-sepharose, hyperactivated LipGTC. Residual activity of the enzyme in methanol and Ca^{2+} were 113% and 122%, respectively (Table 1). Immobilization results are shown in Fig. 5. Adsorption-immobilized form of LipGTC was over 10 times more active comparing with the free form of the enzyme. With time activity depleted retaining more than 50 % of the residual activity after 180 min of using it for catalysis of hydrolysis of *p*-NPB.

Table 1. *Effect of different chemicals on the activity of LipGTC lipase.*

Chemical substance (organic solvents, metal ions, detergents)	Relative activity, %
Methanol	113 ± 2.14
N-Hexane	39 ± 3.45
DMSO	20 ± 4.92
Aceton	0
K^+	73 ± 0.65
Li^+	84 ± 0.65
Mn^{2+}	93 ± 4.07
Mg^{2+}	87 ± 1.72
Ni^{2+}	78 ± 2.35
Ba^{2+}	97 ± 3.96
Ca^{2+}	122 ± 1.72
Zn^{2+}	32 ± 1.95
Fe^{3+}	72 ± 2.26
Tween 40	4 ± 1.93
Tween 60	19 ± 1.3
Tween 80	2 ± 3.14
Urea	69 ± 3.1
SDS	0

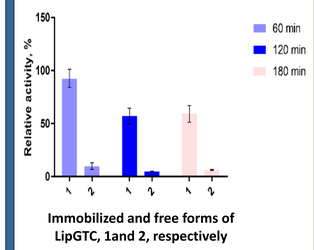


Fig. 5. *Histogram of the activities of immobilized form of LipGTC and the free form of the enzyme at 60, 120 and 180 min after immobilization.*

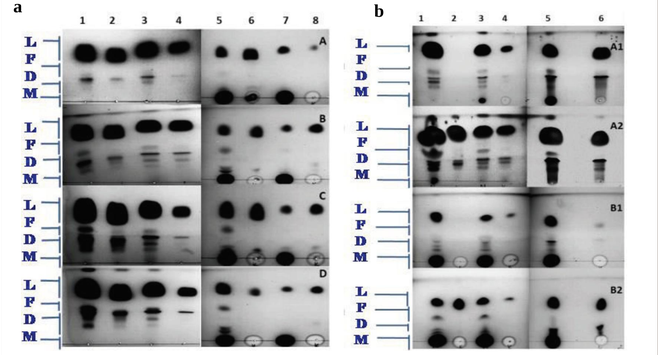


Fig. 4 (a)*. LipGTC specifity toward natural lipid substrates: samples from diethyl ether phase: 1 – hydrolysis of flaxseed, 3 – pumpkin seed oils; 2, 4 – control samples of flaxseed and pumpkin seed oils hydrolysis, respectively. A, B, C, D – incubated for 2, 4, 6, 8 h, respectively. L- lipids, F- fatty acids, D – diacylglycerols, M – monoacylglycerols;* ***(b)*** *1 – hydrolysis of rapseed, 3 – olive, 5 – sunflower oils, 2, 4, 6 – control samples of rapeseed, olive and sunflower oils hydrolysis, respectively. A1, A2 – samples taken from diethyl ether phase after incubation for 2, 4 h, respectively; B1, B2 - samples taken from water phase after incubation for 2, 4 h, respectively L- lipids, F- fatty acids, D - diacylglycerols, M – monoacylglycerols.*

CONCLUSIONS

To conclude, until now there were works published dealing with only one *S. saprophyticus* membrane associated Ssp lipase, therefore, present study widens knowledge about secreted *S. saprophyticus* lipolytic enzymes and emphasizes its potential to be applied industrially.

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