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Biochemical analysis of *Staphylococcus saprophyticus* GTC1 natively secreted lipase

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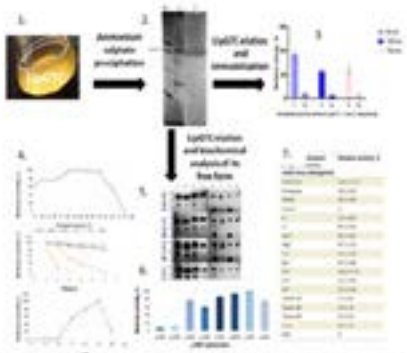
Today microbial lipases find vast applications and have firmly established themselves in diverse areas of industrial microbiology and biotechnology. Among hydrolytic enzymes which are currently published to be the most widely used in industrial processes, lipases represent the third most commercialized 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, 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 a lipase producer was assayed. *S. saprophyticus* GTC1 isolate which was found and identified previously in our work showed remarkable lipolytic activity. As a species it is published to be a part of natural human flora sometimes causing uncomplicated urinary tract infections and is also found in soils. Secretion of the enzyme (LipGTC) was obtained in TSB liquid medium without addition of any inducers. After $(NH_4)_2SO_4$ precipitation, using SDS-PAGE and tributyrin overlay zymography, LipGTC was determined to be ~50 kDa. LipGTC was eluted from the gel and was further analyzed. Enzyme had optimal activity at 35–50°C, pH 9 and hydrolyzed synthetic *p*-NP substrates and different natural lipids releasing highly valuable fatty acids. Some tested chemicals (detergents, different metal ions), as well as immobilization of the enzyme onto octyl-sepharose, hyperactivated LipGTC (relative activity in methanol and Ca^{2+} were 113% and 122%, respectively). LipGTC showed to be highly stable in the range of 30–50°C up to 6 h without remarkable loss of activity. 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.



Scheme of the conducted work in figures: 1. Representation of LipGTC secreted into the medium; 2. SDS-PAGE (1) and zymogram (2) which revealed the molecular mass of LipGTC and were used for the elution of the enzyme (M – molecular marker); 3. LipGTC immobilization onto octyl-sepharose which promoted hyperactivation; 4, 5, 6, 7 – biochemical analysis of the free form of LipGTC: 4 - LipGTC temperature optimum, thermostability and pH optimum (from top to bottom, respectively); 5. Thin layer chromatography of hydrolysis of natural fatty substrates by LipGTC (1 – hydrolysis of flaxseed, 2 – pumpkin seed oils, 3, 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); 6. Histogram of LipGTC specifity towards p-NP substrates; 7. Table showing the effect of different detergents, organic solvents and metal ions on the activity of LipGTC1

Biography

Alisa Gricajeva is currently a PhD student at the Department of Microbiology and Biotechnology, Institute of Biosciences, Life Sciences Center, Vilnius University. Her final thesis work is associated with the study of microbial lipolytic enzymes: search of unstudied enzymes and their activity analysis. She is an author of publications and conference theses dealing with characterization of lipolytic enzymes, improvement of their properties and assumption of their use in biotechnology. She is also a Junior Researcher and Lecturer of Food Microbiology and Biotechnology and Enzymology at Vilnius University.

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