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ORIGINAL ARTICLE

Evaluation of a novel thermo-alkaline *Staphylococcus aureus* lipase for application in detergent formulations

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KEYWORDS

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Abstract An extracellular lipase of a newly isolated *S. aureus* strain ALA1 (SAL4) was purified from the optimized culture medium. The SAL4 specific activity determined at 60 °C and pH 12 by using olive oil emulsion or TC4, reached 7215 U/mg and 2484 U/mg, respectively. The 38 NH_2-terminal amino acid sequence of the purified enzyme starting with two extra amino acid residues (LK) was similar to known staphylococcal lipase sequences. This novel lipase maintained almost 100% and 75% of its full activity in a pH range of 4.0–12 after a 24 h incubation or after 0.5 h treatment at 70 °C, respectively. Interestingly, SAL4 displayed appreciable stability toward oxidizing agents, anionic and non-ionic surfactants in addition to its compatibility with several commercial detergents. Overall, these interesting characteristics make this new lipase promising for its application in detergent industry.

Abbreviations: HPLC, high-performance liquid chromatography; NaDC, sodium deoxycholic acid; NaTDC, sodium taurodeoxy cholic acid; OD, optical density; PCR, polymerase chain reaction; rDNA, ribosomal deoxy ribo nucleic acid; rpm, revolutions per minute; SHyL, *Staphylococcus hyicus* lipase; SAL, *Staphylococcus aureus* lipase; SEL, *Staphylococcus epidermidis* lipase; SL1, *Staphylococcus* sp. lipase; SSL, *Staphylococcus simulans* lipase; SXL, *Staphylococcus xylosus* lipase; *S. aureus*, *Staphylococcus aureus*; SDS, sodium dodecyl sulfate; TFA, tri fluoroacetic acid; TC3, tripropionin; TC4, tributryin; TC8, trioctanoin; TC18, triolein

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1. Introduction

Lipases are water-soluble enzymes regulating the hydrolysis of ester bonds in insoluble acylglycerols at lipid-water interface (Jaeger et al., 1999). They are serine hydrolases composed of Ser-Asp/Glu-His. Besides, a consensus sequence (Gly-x-Ser-x-Gly) that is usually found encircling serine, the active site (Pleiss et al., 2000). Recently, new lipolytic enzymes have shown to have a different pattern (Gly-Asp-Ser-Leu) (Akoh et al., 2004). Lipases three-dimensional (3-D) structures reveal the α/β-hydrolase fold (Nardini and Dijkstra, 1999). Indeed, the active site in most lipases cannot be reached due to the coverage by surface loops or helical structures. The structures of lipases binding substrate and those inhibited by transition-state analogs (Egloff et al., 1995; Hermoso et al., 1996) revealed that when the active site interacts with micelles or substrate molecules, it becomes exposed to substrate. These findings have given a structural basis of the interfacial activation compared to nearly forty years ago (Sarda and Desnuelle, 1958; Brzozowsky et al., 1991). In addition to their carboxylic ester bond hydrolysis, lipases also catalyze the reverse esterification, amidation or transesterification processes in aqueous and anhydrous organic solvents (Bornscheuer and Kazlauskas, 1999).

Microbial Lipases are lately of great commercial importance thanks to their high versatility and high stability as well as the advantage of being readily produced in high yields (Hasan et al., 2009). In fact, there has been now more interest in studying enzymes from extremophiles, not only due to their thermo stability but also to their high resistance to chemical agents and extreme pH values besides their mesophilic homologs (Sharma et al., 2001; Jaeger and Eggert, 2001). Some lipases from fungi and bacteria were exploited as cheap and versatile catalysts in various industries, such as food, dairy, detergent and pharmaceutics, along with the degradation of fatty wastes and biodiesel production (Gupta et al., 2004; Liu et al., 2008).

In 1994, the recombinant lipase "Lipolase", isolated from the fungus *Thermomyces lanuginosus* and expressed in *Aspergillus oryzae* was first introduced by NovoNordisk. Then In 1995, two bacterial lipases 'Lumafast' from *Pseudomonas mendocina* and 'Lipomax' from *Pseudomonas alcaligenes* correspondingly were introduced by Genencor International (Jaeger and Reetz, 1998). Detergent lipases also were originated from *Candida* (Novak et al., 1990) and *Chromobacterium* (Nawani et al., 1998). As for Laundering, that is generally carried out in alkaline media, microbial lipases were found to be active too (Satsuki and Watanabe, 1990; Handelsman and Shoham, 1994).

An increased interest of recently isolated staphylococcal lipases results from their potential in modern biotechnology. The immobilized lipases are used in non aqueous media as biocatalyst to catalyze the alcoholysis, the transesterification, and the esterification of the alcohols with organic acids in various industries (Horchani et al., 2012).

However, thermostable and alkaline tolerant lipases extracted from *Staphylococcus* sp. have been rarely described in the literature (Cherif et al., 2011; Chauhan et al., 2013). Furthermore, their practical application is somehow limited due to their relatively lower stabilities and catalytic activities under high temperature and extreme pH values particularly. Since each application has specific biocatalytic properties related to specificity, stability, optimal temperature, and pH-dependence, there is still an interest in researching new lipases with novel applications.

In our previous study, we have screened, isolated and optimized the production of extracellular lipase by a new *Staphylococcus aureus* (*S. aureus*) strain ALA1 (GenBank no. KF 678862) from dromedary milk (Ben Bacha et al., 2015). Here, we reported the purification and the characterization of a thermoactive, alkaline and detergent-stable lipase (SAL4), as well as its stability in the presence of several commercial solid and liquid detergents, oxidizing agents and surfactants.

2. Materials and methods

2.1. Production and purification of SAL4

S. aureus strain ALA1 lipase was produced as previously reported by Ben Bacha et al. (2015). Cells were discarded after 30 h of culture by centrifugation (30 min, 12,000 rpm) and the resulting crude enzyme solution (250 mL) was precipitated with solid ammonium sulfate (65% saturation) at 4 °C. The precipitate obtained after centrifugation was then resuspended in 25 mM Tris–HCl, pH 8 containing 50 mM NaCl and 2 mM benzamidine (buffer A) and treated for 15 min at 70 °C. After centrifugation for 30 min at 12,000 rpm, the treated supernatant was (7.5 mL, 22040U) dialyzed overnight at 4 °C against buffer A.

The obtained sample was then loaded on a C18 HPLC column pre-equilibrated with 0.1% TFA. It was then eluted with a pure acetonitrile linear gradient 0–80% at a flow rate of 1 mL/min. The lipase activity was checked as previously described and the elution profile of proteins was monitored at 280 nm. After 15%-SDS–PAGE analysis, pure and active fractions were stored at −20 °C until used for more biochemical characterization.

2.2. Determination of lipase activity

Lipolytic activity was tested titrimetrically with a pH-stat using tributyrin (TC4) or olive oil emulsion at 60 °C and pH 12 (Rathelot et al., 1981). Enzyme activity was also measured using tripropionin (TC3) as substrate to investigate the interfacial activation of the pure SAL4 while trioctanoin (TC8) and triolein (C18) were used for its kinetic studies (Abdelkafi et al., 2009). Lipase activity was expressed in international units (U) where 1 U is 1 μmol of fatty acid produced/minute.

2.3. Protein analysis

Protein content was determined following the Bradford protocol (Bradford, 1976). The purified lipase was analyzed electrophoretically by SDS–PAGE (15%) according to the of Laemmli method (Laemmli, 1970). The N-terminal amino acid sequence was identified by automated Edman's degradation (Hewick et al., 1981).

2.4. pH and temperature effects on SAL4 stability and activity

Lipase activity was measured at 60 °C and at different pH (8–13) using appropriate buffers. The pH stability of the enzyme

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