

Review Article

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A Review Paper on Properties of Fungal Lipases

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ABSTRACT

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Enzymes are believed catalysts of nature. Lipases are multifaceted enzymes that are utilized broadly. Lipases exist broadly in nature, but only microbial lipases are industrially important. In last few years, fungal lipases gained important consideration in the industries because of their specificity to substrate and stability under different physical and chemical situations. Fungal enzymes are secreted extracellularly and they can be purified simply, which considerably decreases the cost and makes this source superior over bacteria. The several applications of lipases include hydrolysis of oils and fats, flavor development in food processing, modification of fats, resolution of racemic mixtures, removal of oil stains from clothes, estimation of amount of triacylglycerol in the person serum, removal of pitch from timber during paper manufacturing and chemical analysis. An elaborated study of lipase properties makes possible its application in appropriate industrial processes. So by keeping the huge applications of lipases in mind, the current review is focused on properties such as activity and stability profile in various pH and temperature, impacts of metal ions and stability in organic solvents.

Introduction

Lipases have appeared as one of the important biocatalysts with definite potential for contributing to the billion dollar underexploited lipid technology bio-industry and have been utilized in *in situ* metabolism of lipid and *ex situ* versatile industrial applications (Sharma and Kanwar, 2012). Lipases are triacylglycerol acylhydrolases that catalyze the hydrolytic cleavage of ester bonds present within the triacylglycerol into fatty acids and glycerol. They usually show additional activities such as cutinase, phospholipase, cholesterol esterase, isophospholipase, amidase and other

esterase kind of activities (Svendensen, 2000). Lipases are excreted by every class of living organisms but microbial lipases have gained special attention of industries because of their simple extraction procedures, unlimited supply, capability towards extremes of pH, temperature, metal ions, organic solvents, and chemo-, region-, and enantioselectivity (Thakur, 2012). The lipases are as imperative industrially as the amylases and proteases.

Amongst the different microorganisms identified as source of lipases, filamentous

fungi are believed as the excellent source of extracellular lipase for mass production at industrial level. High cost of lipase production is a main problem in its application in industrial processes. Thus, a variety of efforts have been made to decrease its production cost (Smaniotto *et al.*, 2012). Fungi are generally chosen as lipase producers since they produce extracellular enzymes that can be simply separated from the fermentation media (Maia *et al.*, 1999). Mainly, species belonging to *Penicillium*, *Mucor*, *Rhizopus*, *Aspergillus* and *Geotrichum* are broadly identified as excellent source of lipase (Carvalho *et al.*, 2005; Contesini *et al.*, 2010). Amongst them numerous species of *Aspergillus*, isolated from terrestrial sources, have been documented to excrete lipase with notable properties fit for biotechnological applications (Basheer *et al.*, 2011; Sharma *et al.*, 2016). Physico-chemical properties of several extracellular fungal lipases have been determined. Numerous lipases have been carefully purified and characterized in terms of their stability and activity profiles relative to temperature, pH, and impacts of metal ions, organic solvents and chelating agents (Jayaprakash and Ebenezer, 2012). Therefore, the present review is focused on properties of microbial lipases.

Characterization of extracellular lipases

Some exclusive features of lipases such as their substrate specificity, pH and temperature dependency, stability in organic solvents, activity in metal ions and nontoxic nature leads to their main role in the food processing industries (Verma *et al.*, 2012). The most preferred characteristics of the lipases are their capability to use all mono-, di-, and tri-acylglycerides as well as the free fatty acids in transesterification, little inhibition by product, high activity/yield in non-aqueous media, little reaction time,

resistant to altered pH, temperature, organic solvents and metal ions (Kumar *et al.*, 2012a). Sumathy *et al.*, (2012) have reported that lipase purified from *A. niger* had molecular weight of 40 kD.

Impact of temperatures on activity and stability of lipase

Falony *et al.*, (2006) investigated impact of various temperatures (20-90 °C) on the activity of lipase by *A. niger*. Enzyme was highly active at 40 °C and then lipase activity was started to decline drastically after 60 °C. Enzyme activity was completely lost at 90 °C. Sarkar and Laha (2013) reported that lipase of *A. niger* exhibited maximum activity (0.40 U ml⁻¹) at 40 °C. Jayaprakash and Ebenezer (2012) studied influence of various temperatures (20-90 °C) on the activity and stability of lipase purified from *A. japonicus*. An optimum temperature of 40 °C was found, that was followed by reduction in the lipase activity with rise in temperature and activity reached to minimum at 90 °C. In the stability pattern, lipase remained stable in the temperature range of 30-60 °C when pre-incubated for 1 h. Ulker *et al.*, (2011) depicted an optimum temperature of 40 °C for the maximum activity of lipase from *T. harzianum*. Lipase activity was reduced by changing the temperature optima, while in the stability profile, enzyme remained stable at the temperature range of 20-40 °C, after pre-incubation for 1 h. However activity of lipase was completely lost at high temperature range from 60-80 °C.

Pera *et al.*, (2006) demonstrated activity of *A. niger* lipase within the temperature range of 4-55 °C. Among all temperatures, optimum activity was found at 37 °C while in the temperature stability profile, enzyme remained stable in the temperature range of 30-55 °C with highest stability at 37 °C

when pre-incubated for 1 h. Similarly, an optimum temperature of 37 °C for activity of lipase has been documented by other workers (Kamini *et al.*, 1998; Saxena *et al.*, 2003). However, Essamri *et al.*, (1998) manifested that lipase of *R. oryzae* exhibited optimum activity at 30 °C. Kalindhi and Vijayalakshmi (2015) reported that purified lipase of fungus *E. ashbyii* demonstrated optimum activity at 30 °C with pH of 7.0.

Maia *et al.*, (1999) revealed an optimum temperature of 25 °C for activity of lipase by *F. solani*. Lipase activity was decreased above the optimum temperature and reached to zero at 60 °C while in the stability pattern, highest stability was detected in the temperature range of 25-30 °C after pre-incubation for 1 h. Enzyme became inactivated at temperature above 40 °C. Shu *et al.*, (2006) reported that lipase of *Antrodia cinnamomea* retained stability within the temperature range of 25-60 °C with maximum stability at 45 °C. Ranjitha *et al.*, (2009) reported that the lipase of *V. fischeri* retained 80% of its activity at 35 °C, but the lower residual activities were found at 5 °C, 10 °C and 50 °C. Kumar *et al.*, (2012b) demonstrated that purified lipase of *B. pumilus* RK31 was found stable at 40 °C, 50 °C and 60 °C retaining the 66%, 66% and 69% residual activities.

Impact of pH on lipase activity and stability

Falony *et al.*, (2006) studied influence of various pH on the activity of *A. niger* lipase. In the activity pattern, highest lipase activity was obtained at pH 6.0 among the all pH (4.0-10.0). In the stability profile, the lipase was stable and retained 100% of its activity within the pH range of 4.0 to 7.0 for 24 h. Lipase stability was declined after pH 7.0 and reached to minimum at pH 10.0. Similar results were obtained by Sarkar and Laha

(2013). Pera *et al.*, (2006) depicted that lipase of *A. niger* MYA 135 was active within the pH range of 2.0-10.0 but optimum activity was obtained at pH 6.5, while in the pH stability pattern, lipase retained its activity within the pH range of 2.0-10.0 when pre-incubated for 1 h at 37 °C. Sugihara *et al.*, (1988) previously reported that the lipase secreted by *A. niger* demonstrated its highest activity between pH 4.5 to 5.5 at 25 °C and retained its stability in the pH range from 3.0 to 10.5 at 30 °C for 24 h. For maximum activity of lipase, an optimum pH of 2.5 for *A. niger* (Mahadik *et al.*, 2002), pH of 6.5 for *A. niger* (Kamini *et al.*, 1998), and pH of 9.0 for *A. carneus* (Saxena *et al.*, 2003) has been reported.

Jayaprakash and Ebenezer (2012) investigated influence of different pH (3.0-12.0) on the activity and stability of lipase purified from *A. japonicus*. In the activity profile, pH 7.5 was the best for highest activity of lipase followed by reduction in the activity with rise in the pH and the activity was completely lost at pH 12.0. In the stability profile, the enzyme retained stability in the pH range of 6.5-8.0 when incubated for 24 h. Costa and Peralta (1999) earlier manifested that optimum temperature and pH for activity of lipase by *P. wortmanii* were 45 °C and 7.0, respectively. An optimum temperature and pH for the activity of *A. oryzae* lipase were found to be 30 °C and 7.0, respectively (Toida *et al.*, 1995).

Maia *et al.* (1999) reported that among the all tested pH (6.0-9.0), optimum activity of lipase by *F. solani* was found at pH 8.6. Lipase activity was decreased in the pH values below 8.5 and reached to minimum at 6.5, indicating alkaline nature of lipase. In the pH stability profile, lipase retained 80% of its activity in the pH range of 7.2-8.6 with the highest stability at pH 7.2 when pre-

incubated for 1 h. Ulker *et al.*, (2011) reported that pH 8.5 was found to be the excellent for maximum activity of lipase by *T. harzianum*. Lipase activity was declined by changing the pH above or below the pH optima.

In case of lipase stability, the enzyme was found highly stable at pH optima and retained 70% of its activity within the pH range of 8.0-11.0, after pre-incubation of 24 h. Hoshino *et al.*, (1992) also demonstrated highest stability of *F. oxysporum* lipase at alkaline pH. Shu *et al.*, (2006) reported that lipase of *Antrodia cinnamomea* was found stable within the alkaline pH range of 7.0-10.0 with maximum activity at pH 8.0.

Both the enzyme activity and stability were declined considerably in the pH values above 10.0. An optimum activity of *R. oryzae* lipase within the alkaline pH range has been documented by Minning *et al.*, (1998). Costa-Silva *et al.*, (2014) reported that activity of extracellular lipase of fungus *Cercospora kikuchii* was not lost when kept in the pH range of 3.0-9.0.

Amoozegar *et al.*, (2008) and Kasana *et al.*, (2008) reported stability of lipase enzyme within the pH range of 7.5-8.0. The enzyme also retained 90% of its activity. Ranjitha *et al.* (2009) reported maximum stability (residual activity) of purified bacterial lipase at pH 8.0. Kumar *et al.*, (2012c) revealed highest and lowest activity of purified lipase by *Bacillus* sp. HPE 10 at pH 6.0 and pH 8.0, respectively. Other lipases exhibited stability in the pH range of 5.5 to 9.0 (Fox and Stepaniak, 1983).

Zhang and Zhang (1982) reported that the purified lipase preparation retained stability within the alkaline range of pH from 7.0 to 10.0 justified it to be a potent alkaline lipase in the degreasing process in leather industry.

Impact of organic solvents on stability of lipase

Pera *et al.*, (2006) studied impact of various water miscible solvents (methanol, ethanol, acetone, butanol, hexane and heptane) on the stability of *A. niger* lipase. The enzyme was found stable in all organic solvents with highest residual activity in acetone when pre-incubated for 1 h at 37 °C. Lowest residual activity was obtained with heptanes. Jayaprakash and Ebenezer (2012) investigated influence of different organic solvents (at a concentration of 10% and 20% v/v) on the stability of purified lipase of *A. japonicus*. The enzyme retained 90% of its activity in methanol, acetone, chloroform, ethanol and hexane with highest residual activity (95%) in methanol (10% v/v) when pre-incubated for 1 h. Reduction in lipase stability was noticed with the rise in concentration of organic solvents from 10% to 20%. It was reported by Maia *et al.*, (1999) that lipase of *F. solani* retained 30% of its activity in acetone and n-propanol (10% v/v) while 20% concentration of both solvents completely suppressed lipase stability.

However, Zhou *et al.*, (2012) reported inhibition of lipase activity by ethanol and n-butanol. It was reported that organic solvents (water miscible) shred water from the enzymes, which results in denaturation of the molecule at a much rapid rate than in a pure water system (Azevedo *et al.*, 2001). Kumar *et al.*, (2012b) reported highest (120.50%) and lowest relative activity (10%) by *B. pumilus* RK31 in petroleum ether and diethyl ether, respectively.

Impact of metal ions on stability of lipase

Jayaprakash and Ebenezer (2012) investigated impact of different metal ions

(1 mM) on stability of lipase purified from *A. japonicus*. Activity of lipase was inhibited by Mn^{2+} and Hg^{2+} while Ca^{2+} was found to be the best for maximum activity after pre-incubation for 1 h. Ulker *et al.* (2011) reported stability of *T. harzianum* lipase after pre-incubation for 1 h in various metal ions (1 mM). Ca^{2+} and Mn^{2+} increased the activity of lipase up to 25% and 15%, respectively, while K^+ and Cr^{3+} inhibited the lipase activity by 22% and 21%, respectively as compared with the control. Lipase activity was not influenced by other metal ions used in the study (Na^+ , Ba^{2+} , Cu^{2+} , Cd^{2+} , Co^{2+} and Fe^{3+}). Katiyar and Ali (2013) reported highest increase in catalytic activity of *Candida rugosa* lipase by Ca^{2+} . This might be due to the fact that the enzyme requires Ca^{2+} as a cofactor for its biological activity.

Kambourova *et al.*, (2003) described that the stimulatory impact of Ca^{2+} is because of formation of insoluble ion-salts of fatty acids during hydrolysis, hence avoiding the inhibition of product formation. Yu *et al.* (2009) depicted that activity of lipase from *R. chinensis* was enhanced up to 24% by Ca^{2+} (1 mM) while on the other hand, Ohnishi *et al.* (1994) reported that activity of *A. oryzae* lipase was inhibited up to 77% by Ca^{2+} (5 mM).

Toida *et al.*, (1995) reported inhibition of activity of *A. oryzae* lipase by Cu^{2+} , Fe^{3+} , Hg^{2+} , Zn^{2+} and Ag^+ . Oliveira *et al.* (2014) reported that Na^+ increased lipase stability of yeast by 5.6%. Ghori *et al.* (2011) demonstrated that activity of *Bacillus* sp. lipase was enhanced by Mg^{2+} , Mn^{2+} and Fe^{2+} while Co^{2+} , Cu^{2+} and Na^+ decreased the lipase activity. Tiwari *et al.* (2011) reported that lipase activity was increased in presence of Mg^{2+} , Ba^{2+} and Ca^{2+} while it was decreased in presence of Ag^+ . It was reported by Costa-Silva *et al.* (2014) that the ions Al^{3+} , Ca^{2+} , Mn^{2+} , Zn^{2+} and Hg^{2+}

increased the extracellular lipase activity of the fungus *C. kikuchii*. Residual lipase activity was increased to 129.30% in presence of Al^{3+} ion as compared to control.

Hasan *et al.*, (2006) had previously described that metal ions have tendency to make complexes with ionized form of fatty acids, which results in changing their behaviour and solubility at interfaces. The liberation of fatty acids into the culture medium is rate determining factor, which is influenced by metal ions. However, the impact of metal ions varies and depends on the type of lipase. The activity of extracellular lipase from *R. japonicus* NR400 was not influenced in the presence of metal ions (1 mM) (Suzuki *et al.*, 1986). Mase *et al.* (1995) reported that activity of lipase by *P. roqueforti* IAM7268 was not influenced by the addition of Ca^{2+} , Mg^{2+} , Mn^{2+} , Na^+ , K^+ and Cu^{2+} .

In conclusion, fungi are able to produce many enzymes for their continued existence within a broad variety of substrates. Among those enzymes, lipases are mainly utilized in a number of applications. The major benefit of fungal lipases is that they are simply acquiescent to separation because of their extracellular nature, which considerably decreases the overall cost and makes these lipases more interesting than bacterial lipases. A detailed characterization study (activity and stability under different pH, temperature, metal ions and organic solvents) of fungal lipases should be done in order to determine their suitability in various industrial processes.

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