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Bacterial lipases: A review on purification and characterization

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ABSTRACT

Lipase (E.C.3.1.1.3) belongs to the hydrolases and is also known as fat splitting, glycerol ester hydrolase or triacylglycerol acylhydrolase. Lipase catalyzes the hydrolysis of triglycerides converting them to glycerol and fatty acids in an oil-water interface. These are widely used in food, dairy, flavor, pharmaceuticals, biofuels, leather, cosmetics, detergent, and chemical industries. Lipases are of plant, animal, and microbial origin, but microbial lipases are produced at industrial level and represent the most widely used class of enzymes in biotechnological applications and organic chemistry. Phylogenetic analysis and comparison of residues around GxSxG motif provided an insight to the diversity among bacterial lipases. A variety of para-Nitrophenyl (p-NP) esters having C_2 to C_{16} (p-NP acetate to p-NP palmitate) in their fatty acid side chain can be hydrolyzed by bacterial lipases. Large heterogeneity has been observed in molecular and catalytic characteristics of lipases including molecular mass; 19–96 kDa, K_m; 0.0064–16.58 mM, K_{cat}; 0.1665–1.0 × 10^4 s^{-1} and K_{cat}/K_m; 26.02–7377 s^{-1}/mM. Optimal conditions of their working temperature and pH have been stated 15–70 °C and 5.0–10.8, respectively and are strongly associated with the type and growth conditions of bacteria. Surface hydrophobicity, enzyme activity, stability in organic solvents and at high temperature, proteolytic resistance and substrate tolerance are the properties of bacterial lipases that have been improved by engineering. Bacterial lipases have been extensively studied during last decade. However, their wider applications demand a detailed review on purification, catalytic characterization and applications of lipases.

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1. Introduction

Significant rising concern in the field of enzymology on account of wider applications of enzymes in various chemical processes has been increased since a few decades (Pliego et al., 2015). Due to versatile applications, lipases are the third most abundantly used enzymes after proteases and amylases (carbohydrases) (Ülker et al., 2011). Lipase (E.C.3.1.1.3) is also known as fat splitting, glycerol ester hydrolase or triacylglycerol acylhydrolase and belongs to the class of enzymes that catalyze the hydrolysis reactions (hydrolases). Lipase catalyzes the hydrolysis of triglycerides converting them to glycerol and fatty acids in an oil-water interface. Lipases also have a property to reverse this reaction in an aqueous and non-aqueous media (Faouzi et al., 2015b; Lee et al., 2015; Priji et al., 2015; Ramos-Sanchez et al., 2015; Ullah et al., 2015). Some lipases show enantioselective properties and used to catalyze the processes of esterification, interesterification, transesterification, acidolysis and aminolysis (Hasan et al., 2009). The substrates of lipases (long chain triacylglycerols) are insoluble in water, hence, these are first dissolved in organic solvents followed by mixing with buffer (two-phase system). However, lipases are soluble in water and can catalyze their reactions in two types of systems including aqueous and organic medium. Organic solvents may denature and cause conformational changes in the lipase structure and hence influence their functional and catalytic activities (Guo et al., 2015).

Lipases are ubiquitous enzymes (Priji et al., 2015), belong to α/β hydrolase fold super-family (Kapoor and Gupta, 2012) and have a network of hydrogen bonds at their active site containing triad of Ser, Asp (Glu) and His (Faouzi et al., 2015b; Farrokh et al., 2014; Thakur et al., 2014). Lipase catalyzed transesterification for the production of biodiesel is an efficient, energy-saving, and environment friendly process and is a promising alternative to the conventional chemical catalysis (Fjerbaek et al., 2009). Lipases are substrate specific enzymes and have properties like chemo-, region-, stereo-specificity and ability to catalyze heterogeneous reactions both in water soluble and water insoluble systems. On account of their wider catalytic properties, lipases are extensively used as biocatalysts in different industries like agrochemical, pharmaceutical, detergent, tanning, food and surfactant producing industries (Ananthi et al., 2014; Iftikhar et al., 2012; Kumar et al., 2012a,b; Thakur et al., 2014).

On the basis of positional specificity (regiospecificity), lipases are divided into three classes.

(i). Non-specific lipases

These lipases catalyze the triglyceride into free fatty acids and glycerol with mono- and di-glycerides as intermediates and can remove fatty acid from any position of the substrate. Mono- and diglycerides are hydrolyzed more rapidly than triglyceride. (Kapoor and Gupta, 2012; Ribeiro et al., 2011).

(ii). 1, 3-specific lipases

These lipases release fatty acids from position 1 and 3 of the triglycerides and cannot hydrolyze ester bonds at secondary positions. Hydrolysis of triglycerides by 1, 3-specific lipases to diglycerides is much faster than those into mono glycerides (Kapoor and Gupta, 2012; Ribeiro et al., 2011).

(iii). Fatty acid-specific lipases

A third group of lipases shows fatty acid selectivity and catalyzes the hydrolysis of esters which have long-chain fatty acids with double bonds in cis position between C-9 and C-10 (Kapoor and Gupta, 2012; Ribeiro et al., 2011).

Lipases need no co-factor for their activity and remain active in organic solvents (Lee et al., 2015; Ullah et al., 2015). Consumption of all monoglycerides, diglycerides, triglycerides and free fatty acids in the process of transesterification, high production in non-aqueous media, low reaction time and resistance to low pH are some of the properties which make lipases more desirable biocatalysts (Ashfaq, 2015). Lipases are of plant, animal, and microbial origin, but microbial lipases are produced at industrial level and represent the most widely used class of enzymes in biotechnological applications and organic chemistry due to higher catalytic activity, seasonal changes independent production, ease in genetic manipulation for desired characteristics, production in bulk quantity and use of cheaper growth culture media (Dey et al., 2014; Lee et al., 2015; Priji et al., 2015; Ullah et al., 2015).

Ease of genetic and environmental manipulation is very beneficial for the production of microbial lipases in a way that this allows us to produce altered enzyme with a variety of catalytic activities. Bacterial lipases may be intracellular, extracellular or attached to membrane. Extensive work has been done on various aspects of lipase production from various sources and their applications. Multiple studies on physico-chemical and catalytic properties have also been shared. However, a comprehensive review on multiple aspects of lipases is direly needed to sum up the developments happen in the field so far. Current review provides recent and detailed information about production, purification, characterization, phylogenetic analysis, engineering and applications of bacterial lipases.

2. Lipase producer bacterial strains

Lipase from bacterial sources is considered more suitable to withstand the hardy industrial environment. A major part of the work on the production and characterization of lipases has been focused from bacterial sources. The bacterial strains reported in the literature for the production of lipases are summarized in Table 1.

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