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Review

Solvent tolerant lipases: A review

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ABSTRACT

Lipases have proven to be useful in different hydrolytic and synthetic reactions of industrial importance. Microbial strains from natural and extreme environments produce lipases with unique characteristics. The ability of lipase to withstand different environmental conditions during reactions, including temperature and pH, is essential. Solvent systems tend to affect lipase-catalyzed reactions, and thus the careful selection of both the medium and the lipase source is necessary. This review considers different solvent systems used in lipase-catalyzed reactions and some of the enzymatic properties required for function. Other properties of interest besides enzyme activity include tolerance, stability and compatibility to different reaction media, such as acid, alkaline, salt, organic solvents and other compatible solvents (ionic liquids and detergents). For lipase to be used in a detergent, its thermostability and alkaline tolerance must be well pronounced. In addition, organic solvent stability plays an essential role in employing lipases for biodiesel production. Thus, the selection of the lipase for each application is based on specificity and stability in different solvent systems, which gives lipases many potential applications in aqueous and non-aqueous biocatalysis.

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Contents

1. Introduction

Lipases are triacylglycerol ester hydrolases (EC 3.1.1.3) that have a strong ability to catalyze both hydrolytic and synthetic reactions in nature. These properties allow them to be employed in different biochemical reactions, including esterification, transesterification, interesterification, acidolysis, aminolysis, alcoholysis, acylation, and resolution of racemates [1,2]. The boundless application potential of lipases is apparent in the production of biofuels, organic synthetic compounds, detergents, perfumes, cosmetics, leather, enantiopure pharmaceuticals, medical diagnostics, foods and feeds [3,4]. The selection of lipase for each of these applications is based on its specificity and stability in different solvent systems.

Microbial sources from fungi, bacteria and archaea produce lipases with unique features that can be used for biotechnological applications. However, microbial lipases are not comparable with plant and animal lipases in terms of activity, yield, ease of

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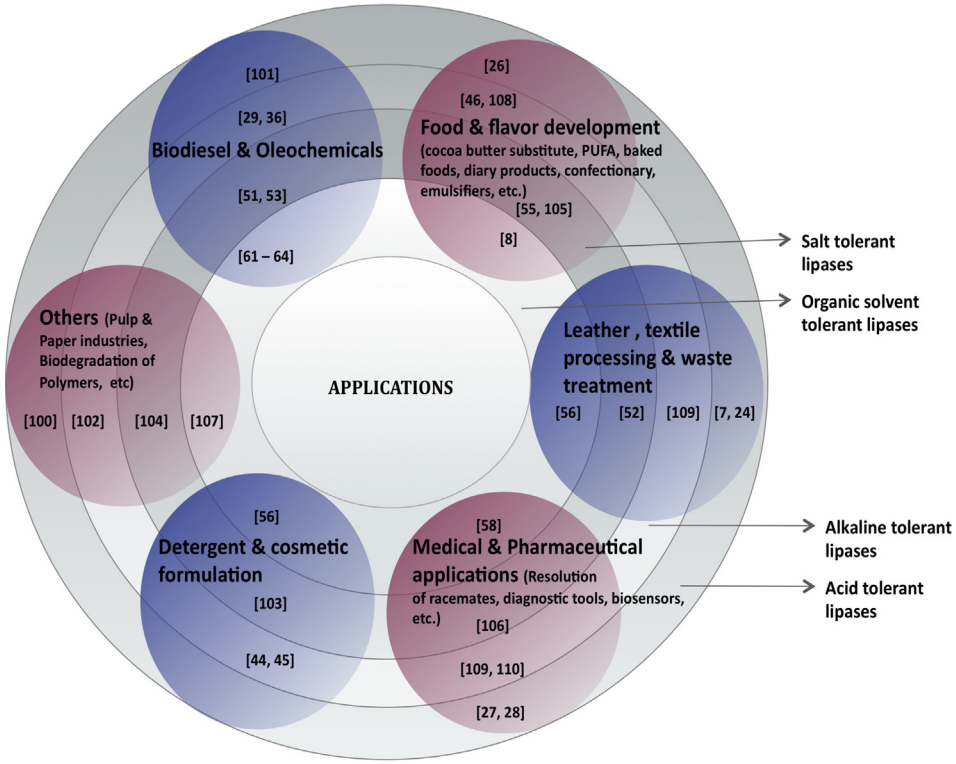

Fig. 1. Application of lipases in different solvent systems. Each line within the main circle represents a particular solvent system, and the number(s) in parenthesis indicate reference(s) [100–110].

purification and molecular modifications, and continuous production independent of season and stability [5,6]. The environment where these organisms are isolated occasionally confers some characteristics to the enzymes. Lipases isolated from thermophilic, halophilic, alkaline and acidic sources tend to be thermostable, salt, alkaline and acid tolerant, respectively. *Bacillus* sp. isolated from soil obtained around edible oil and fish oil processing plants have acidophilic characteristics, with an optimum pH of 1 [7]. Lipase from *Acinetobacter* sp. EH28, which was isolated from oil spill sites, is thermostable with good tolerance to alkaline and organic solvents [8]. In the case of salt tolerance, Ozcan et al. [9] reported that archaeal strains have halophilic properties. Thus, lipases are employed in various processes of industrial relevance, as shown in Fig. 1.

Lipases with high activity in aqueous media tend to have lower activities of up to four or five orders of magnitude in non-aqueous media. Several factors may contribute to this effect, including disruption of tertiary structure due to changes in medium hydrophobicity, limited conformational flexibility, desolvation of the active site resulting in limited enzyme-substrate binding and interfacial denaturation of the enzyme due to interfacial tension [10,11].

Lipases with enhanced catalytic potentials are sought for in different applications. Microbial wild strains often show good characteristics, but using them directly for lipase biocatalytic reactions is impracticable due to low yield and deactivation. Molecular techniques that involve the cloning and expression of foreign proteins in appropriate eukaryotic systems have been extensively used for enhanced lipase catalysis [12]. Other approaches known to enhance the catalytic efficiency of lipases are associated with solvent engineering and molecular imprinting, directed evolution and mutagenesis [13,14].

This article presents an overview of solvent tolerant lipases by describing different solvent systems for lipase-catalyzed reactions ranging from acid, alkaline, salt and organic solvents. Each solvent system has unique features that make lipases versatile in their biocatalytic transformations.

2. Acid tolerant lipases

Acid tolerant lipases have the potential to be utilized for wastewater treatment, leather processing and medical applications. In the food industry, acidic lipases contribute greatly to the synthesis of aroma- and flavor-containing compounds [15].

Lipase-producing microorganisms undergo several adaptive processes to circumvent the effect of an acidic environment, including the selective permeability of the membrane to protons, fortification of macromolecular structures, production of chaperonins and other acid shock proteins such as DnaK, GroEL and HtrA, changes in membrane fatty acid saturation dominated by monounsaturated fatty acids and longer chain fatty acids, and regulation of genes that contribute to membrane stability, composition and activity [16–18]. Thus, the lipases produced by these organisms show unique characteristics at low pH.

Enterococcus durans NCIM5427, isolated from fish visceral waste, produces a lipase that is stable under acidic conditions from pH 2 to 5, with maximum activity occurring at pH 4.6. This finding suggests that the source and the nature of the environment have a great influence on the characteristics of lipase [19]. Using babassu oil cake as a renewable substrate, lipase produced by *Penicillium simplicissimum* (a wild-type Brazilian strain) has maximum activity under acidic pH values. The strain is thermostable, based on its half-life of more than 5 h at 50 °C and pH 5 [2]. *Aspergillus oryzae* CJLU-31 was isolated from soil containing waste cooking oil by Zhou et al. [20]; the acid-tolerant lipase produced by this strain had maximum activity at pH 4 and retained more than 80% of its activity at lower pH values. Additionally, lipase from *Kluyveromyces marxianus* was stable under acidic conditions,

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