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Review

Molecular and functional diversity of yeast and fungal lipases: Their role in biotechnology and cellular physiology

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ABSTRACT

Lipase catalyzes hydrolysis of fats in lipid water interphase and perform variety of biotransformation reactions under micro aqueous conditions. The major sources include microbial lipases; among these yeast and fungal lipases are of special interest because they can carry out various stereoselective reactions. These lipases are highly diverse and are categorized into three classes on the basis of oxyanion hole: GX, GGGX and Y. The detailed phylogenetic analysis showed that GX family is more diverse than GGGX and Y family. Sequence and structural comparisons revealed that lipases are conserved only in the signature sequence region. Their characteristic structural determinants *viz.* lid, binding pocket and oxyanion hole are hotspots for mutagenesis. Few examples are cited in this review to highlight the multidisciplinary approaches for designing novel enzyme variants with improved thermo stability and substrate specificity. In addition, we present a brief account on biotechnological applications of lipases. Lipases have also gained attention as virulence factors, therefore, we surveyed the role of lipases in yeast physiology related to colonization, adhesion, biofilm formation and pathogenesis. The new genomic era has opened numerous possibilities to genetically manipulate lipases for food, fuel and pharmaceuticals.

Contents

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1. Introduction

Lipases (triacylglycerol hydrolase, E.C. 3.1.1.3) are the enzymes that catalyze hydrolysis of triacylglycerides into free fatty acids and glycerol at the lipid water interface [1]. These hydrolytic reactions can be reversed under micro aqueous environment, such as in organic solvents, leading to esterification and transesterification. Lipase research has largely been focused on investigating the broad substrate specificity and regio-, chemo- and chiral-selectivity [2–8]. Owing to their unique properties, lipases are frequently used in various industrial sectors *viz.* detergent formulations, oleochemical industry, biofuels, food and dairy, agro-chemical, paper manufacturing, nutrition, cosmetics and pharmaceuticals [3–10]. Lipases are well known industrial biocatalyst due to their ability to carry out multitude of bioconversion reactions.

According to a report by Business Communications Company, Inc. in 2007, lipases are forecasted as the fastest growing class of enzyme. The potential for an array of applications has driven an outspread search for novel lipases. Multiple groups in the world are working to understand the structural basis of enantio-selectivity, engineer enzyme-specificity and improve technology to enhance production and yield [11,12]. This involves unforeseen application of modern molecular techniques such as site-directed mutagenesis and evolution-mediated generation of radically different and novel biocatalysts [2,13–17].

Lipases are ubiquitous enzymes and are produced by range of organisms including bacteria, yeast, plants to higher organisms. Lipases from bacteria and yeasts are of special interest because of their ease of production and have been reviewed frequently in the past [3,10,18–21]. Major focus has always been on developing the biotechnological applications of bacterial or specific yeast lipases from *Candida rugosa* and *Candida antarctica* [7,8,10,22–25]. However, the sequence and structure based functional prediction of yeast lipases are still poorly described. With the development of high-throughput sequencing approaches, modern genomic databases and advanced bioinformatic tools, there is a paradigm shift towards interdisciplinary approaches for tailoring enzymes. Computational analysis of sequence diversity, structural and functional analysis of proteins are important for predicting mutations for desired catalytic functions, before undertaking experimental trials [26]. This review focuses on all these aspects including phylogenetic analysis, structure comparisons along with functional determinants. Based on the available information, we attempt to identify hotspots in lipases for mutagenesis for developing enzyme variants with improved thermostability and substrate specificity. A brief account on biotechnological applications in few important sectors is also presented. Recently, extracellular lipases have also been reported to play an important role in immunology, adhesion and pathogenesis [27–29]. Hence, the last section of the review highlights the role of lipases in the physiology of yeast with respect to growth, adhesion and biofilm formation.

2. Sources of lipases

Lipases are ubiquitous in nature and are found in multiple unicellular and multicellular organisms. However, yeast and fungi are one of the most important sources of lipases for industrial applications [30]. Most commercially important lipase-producing yeasts belong to the class of ascomycetous yeast, like *Candida* sp. Most of the lipases are extracellular and can be obtained either by submerged fermentation (SmF) or by solid-state fermentation (SSF) [31]. Lipolytic yeasts are found in a variety of oil contaminated habitats including soil contaminated with oil, wastes of vegetable oils, dairy waste and deteriorated food [32]. There are number of lipase producing yeast sources compiled by several authors, however only a few have been commercially exploited for the bulk production [9,33–36]. Some important sources are: *C. antarctica*, *C. rugosa*, *Candida tropicalis*, *Candida curvata*, *Candida cylindraceae*, *Candida deformans*, *Candida parapsilosis*, *Candida utilis*, *Candida valida*, *Candida viswanathii*, *Galactomyces geotricum*, *Arxula adeninivorans*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, *Trichosporon fermantans*, *Trichosporon asahii*, *Rhodotorula mucilaginosa*, and *Aureobasidium pullulans* [34–36]. Recently, few more lipase producing yeasts have been reported such as *Rhodosporidium babjevae* from fresh water of Archipelago, Svalbard [37]; *Candida boidnii* from spent olive fruits of the Algerian variety [38] and *Rhodotoryla slooffiae*, *Rhodotoryla muciloginosa*, *Candida davisiana*, *Cryptococcus diffluens*, *Cryptococcus uzbekistanensis*, *Cryptococcus albidus* and *Wickerhamomyces anomalus* from petroleum sludge [39].

3. Classification of lipases

Lipase classification was first presented for bacterial lipases on the basis of protein topology as lipases have high sequence diversity [40]. According to Arpigny et al., lipases are classified into eight families, out of which six sub families have characteristic α/β hydrolases fold, while other two families II and VIII consist of SGNH-hydrolase and β-lactamase fold, respectively. This classification has been revised several times and currently there are fifteen families that are a part of ESTHER database available on http://bioweb.ensam.inra.fr/esther [41–49]. ESTHER is a broad database, having information on a large diversity of α/β hydrolases fold super family that includes lipases [50]. In addition, a sequence based comparatively simplified version of lipase data is available in lipase engineering database (LED).

LED lists sequences of all the available microbial lipases and provides links to 22 published lipase structures. LED serves as a bioinformatics tool for systematic analysis of sequence relationship, structure and function of diverse lipase proteins, and for designing variants with optimized properties [51]. It was last updated in 2009 and comprises 24,783 sequence entries corresponding to 18,585 proteins as well as 656 experimentally determined protein structures [52]. The classification of lipases in LED database is mainly on the basis of oxyanion hole where lipases are classified into three different classes: GGGX, GX and Y type and further divided into superfamilies on the basis of conserved pentapeptide (Fig. 1).

GGGX class: GGGX class is sub divided into 6 super families that includes 4 super families of lipases and two esterase families *viz.* abH02 – *Y. lipolytica* lipase like, abH03 – *C. rugosa* lipase like, abH04 *Moraxella* lipase 2 like and abH05 – Hormone sensitive lipases having conserved pentapeptides, GHSLG, GESAG, GDSAG and GASAG, respectively.

GX class: GX class consists of 27 super families that includes only 10 super families of lipases and 17 containing lipase like proteins *viz.* abH23 – Filamentous fungi lipases, abH07 – *Moraxella*