

## REVIEW: Novel sources and functions of microbial lipases and their role in the infection mechanisms



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### ABSTRACT

Lipases belong to the family of serine hydrolases, which in turn include various esterase enzymes (E.C.3.1.1.1). They are involved in the cleavage of triacylglycerols to free fatty acids and glycerol in many important biological processes, as for instance routine metabolism of dietary triglycerides to cell signalling and inflammation. Lipases constitute a ubiquitous group of enzymes able to catalyse a number of different reactions, many of them of industrial interest. Particularly, microbial lipases exhibit a wide range of industrial applications, namely in pharmaceutical, food and detergents industry. The aim of this review is to summarize the recent achievements illustrating the importance and the versatility of microbial lipases, including their involvement in infection mechanisms.

### 1. Introduction

Lipases (triacylglycerol acyl hydrolases, E.C. 3.1.1.3) belong to the family of serine hydrolases, which include several esterase enzymes. These enzymes are able to catalyse the hydrolysis of fats and oils in the oil-water interface with the release of free fatty acids, diglycerides, monoglycerides, and glycerol [1,2]. Furthermore, they also have the ability to catalyse esterification reactions, transesterification and interesterification in organic solvents [3,4]. Lipases as catalysts have been reported to play a special and important role in the detergents industry, food production and processing, pharmaceutical, paper, cosmetics and chemical synthesis industries [2,5]. Several studies have been published reporting the different applications of lipases (Table 1). In fact, the use of lipases in the field of biotechnology has been known as a rentable business involving billions of dollars [6]. In the production of cosmetic products, they can function as active ingredients or as biocatalysts in the synthesis of specific cosmetic chemicals, mainly esters, aromatic compounds and active agents [7].

Lipases can split emulsified esters of glycerine and long-chain fatty acids such as triolein (trioleilglycerol, TC18) [8]. For esterases, the substrate is considered the standard tributyrin (tributirilglycerol, TC4), which in turn can be hydrolysed by lipases [9]. It is generally accepted that esterases can hydrolyse ester and triglyceride esters of short carbon chain (< 10 carbons) bonds, releasing fatty acids of low molecular

weight, and therefore are also called non-lipolytic esterases [6] or carboxylesterases [10], since lipases preferentially hydrolyse triglycerides of long chain (> 10 carbons) bonds.

Lipases are widely distributed in nature [3] being found in animals, such as pancreatic lipase, lipases of the tongue, milk, adipose tissue, etc.; in plants (mainly in oily seeds); in bacteria, mainly in species of the genera *Chromobacterium*, *Pseudomonas* and *Staphylococcus*; in yeasts, mainly in species of the genera *Candida* and *Yarrowia lipolytica* and in fungi especially in species of the genera *Aspergillus*, *Geotrichum*, *Rhizopus*, *Mucor*, *Penicillium* [3,11].

Lipases are quite diverse regarding their enzymatic properties and substrate specificity, which together with their easy availability makes them very attractive for multiple biological functions and biotechnological applications. Microbial lipases isolated from bacteria and fungi, are the most appealing in biotechnology and organic chemistry, mainly due to their properties, such as the shortest generation time, high yield of conversion of substrate into product, versatility and ease of mass production [3,12].

### 2. Kinetic and physicochemical characteristics

Lipases are usually acidic glycoproteins [29], with a molecular mass between 20 and 60 kDa and the isoelectric point may vary between 4 and 5 [30]. Most lipases have an optimal range of activity and stability

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**Table 1**  
Some fields of industrial applications of microbial lipases.

Application field	Remarks	References
<i>Biotechnological</i>	<ul style="list-style-type: none"> <li>● Catalyse a several reactions (transesterification and etherification), production of antidepressants, anti-hypertensive and vasodilators;</li> <li>● Generate enantiomerically enriched primary and secondary alcohols;</li> <li>● Bioremediation;</li> <li>● It is used for removing the triglyceride waxes industry, removing hydrophobic components that cause problems in paper manufacture.</li> </ul>	[13,14] [15] [16,17] [3,18,19]
<i>Food industry</i>	<ul style="list-style-type: none"> <li>● Flavour enhancement in dairy products such as cheese butter and margarine;</li> <li>● Brewing industry;</li> <li>● Improve flavours of rice and alcoholic beverages;</li> <li>● It is used in tea processing;</li> <li>● Hydrolysis of milk fat; production of substitutes for butter and other additives used in cereals, snacks and chewing.</li> </ul>	[3,18–20] [21] [18] [3,18] [22]
<i>Detergent industry</i>	<ul style="list-style-type: none"> <li>● Enhance the ability to remove tough stains;</li> <li>● Industrial or household cleaners; dishwashing;</li> <li>● Remove fat (lipids) stains.</li> </ul>	[5] [3,18,23] [3,18]
<i>Pharmaceutical and cosmetics</i>	<ul style="list-style-type: none"> <li>● It is an important compound used in the synthesis of anti-inflammatory drugs (ibuprofen and Naxopreno); hair-waving preparation; skin inflammations; slimming down out by fat removal;</li> <li>● Stereo-selectivity</li> <li>● 2-phenyl-propanoic acid, important compound used in the synthesis of anti-inflammatory drugs, it can be obtained by transesterification reactions.</li> </ul>	[20,24,25] [26] [20,24,25]
<i>Medical applications</i>	<ul style="list-style-type: none"> <li>● It is used as digestive aids; treatment of malignant tumours; treatment of gastrointestinal disturbances, dyspepsia, cutaneous manifestations of digestive allergies.</li> </ul>	[3]
<i>Other applications</i>	<ul style="list-style-type: none"> <li>● Leather manufacture (Degreasing of leather);</li> <li>● It is used to improve the recovery of humidity and facilitates to the removal or dissolving fatty stains in textile industry;</li> <li>● Has an enormous advantage in oleo-chemical industry because it saves energy and minimizes thermal degradation during the reactions of hydrolysis, glycerolysis, and alcoholysis</li> <li>● In the biodiesel production catalysed by lipases through transesterification of triglycerides with short-chain alcohols. In this process, different types of oils, such as soybean, sunflower, corn, jatropha, karanja, waste cooking oil, <i>Chlorella protothecoides</i>, <i>Chlorella protothecoides</i>, animal fat, groundnut, canola, coconut and palm oil has been used;</li> <li>● Fine and bulk chemical industries (acrylamide, wastewater treatment, papermaking, pesticide formulation, soil erosion prevention and gel electrophoresis);</li> <li>● For the resolution of Racemic Acids and Alcohols;</li> <li>● In the Enantioselective catalysis;</li> <li>● Biosensors;</li> <li>● Also is used in waste treatment (degradation of organic debris and sewage treatment, cleaning of holding tanks, septic tanks, grease traps, etc.)</li> </ul>	[3,18,19] [3,18,20,21] [23,27] [23,27] [23,27] [28] [20] [1,19] [3,18]

for pH values between 6.0 and 8.0 and temperature between 30 and 40 °C. However, these properties may vary significantly depending on the source, or even between isoforms produced by one microorganism.

Lipases are water soluble enzymes that act on the metabolism and digestion of triacylglycerols [31,32], thus, the reactions catalysed by lipases occur using a lipidic substrate in the form of emulsion. However, they have a better performance when the substrate concentration is sufficiently high to form micellar aggregates or emulsions, as they can interact with the user interface of the aggregate substrate [31]. This increased activity in the presence of micelles or emulsions is due to the phenomenon known as interfacial activation [33], which in lipases has been early observed in studies undertaken with pancreatic lipases of pig [34,35]. The purified lipases allowed the study of the phenomenon in detail, where the lipase activity of triacetin increased remarkably, since it exceeded its limit of solubility.

The model of the pancreatic lipase enzyme was proposed with the assumption that the interfacial activation could answer a conformational change suffered by lipases [36]. These reactions cannot be described by a Michaelis-Menten reaction, since the hydrolysis process has several steps [36]. With respect to the model substrate, the activation is explained with changes in the lipid substrate interface: increasing the local concentration of the substrate instead of the active site, will reduce the orientation and conformation of lipids in the interface states related to the geometry of the active site of the enzyme, or lessen the degree of hydration of the substrate [37,38].

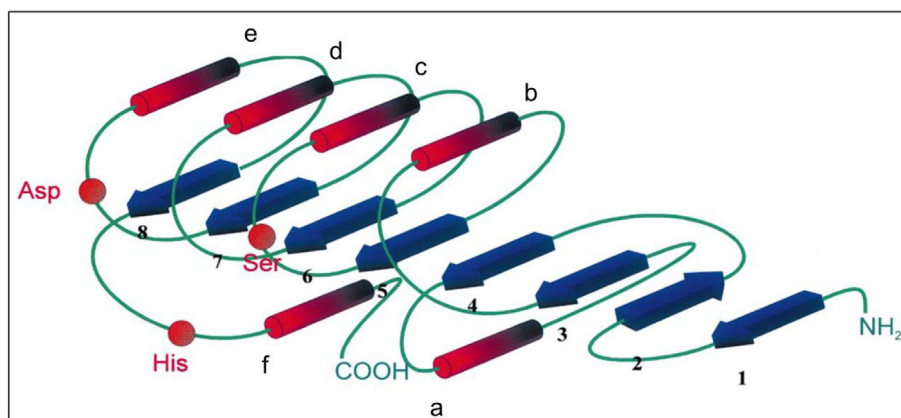
### 3. Structural characteristics

Pleiss and co-workers, have compared the structure of the active site of various esterases and lipases and they observed that those from lipases are normally wider and deeper than the ones from esterases,

which could explain the ability of lipases to recognize a more varied and longer chains substrates (e.g. triolein) [38,39].

When a lipase in the aqueous phase is under its solubility limit or in the absence of/or at low concentration emulsion of a lipid substrate, exists typically a helix covering the active site, in particular, an oligopeptide helix motif termed “lid” which protects the active site. This amphiphilic cover consists of polar amino acids on the outside and nonpolar amino acids on the inner side, in contact with the active site [39]. It has been reported that lipases with the lid covering the active site are in a closed conformation and, in the presence of an emulsion (upon contact with the interface formed by the lipid lipases), undergo a conformational change in the lid region exposing their active site for the hydrolysis of triacylglycerol molecules [40]. The active site is only exposed when the cover opens, i.e. the enzyme only catalyses a reaction in hydrophobic-hydrophilic interfaces or in the presence of a hydrophobic solvent. The mechanism of conformational change from “closed to open” is called interfacial activation [26].

The active site of lipases is generally characterized by a triad of serine, histidine and an acid residue (aspartic or glutamic acid) classified as serine hydrolases, which are essential for all the reactions catalysed by these enzymes [6,41]. The mechanisms involved in the catalysis of serine hydrolases has been previously proposed [41,42]. The first step consists of the removal of a proton from serine, mechanism by which aspartate and histidine residues are required. The hydroxyl group of serine attacks the carbonyl carbon forming an intermediate substrate. The presence of an oxoanionic spacer contributes to the stabilization of the charge distribution and also to reduce the minimum energy for the formation of a tetrahedral intermediate. The last step is the deacylation: the acyl group is transferred to the enzyme and released by the attack of a nucleophile (e.g. H<sub>2</sub>O), thus the catalytic centre of the enzyme is regenerated.



**Fig. 1.** Schematic representation of the structural motif conserved enzyme family of  $\alpha/\beta$  hydrolase. The filaments forming the structure in  $\beta$ -pleated sheet (1–8) are indicated by blue arrows and the structures in  $\alpha$ -helices (A to F) are indicated by red columns. The relative positions of the amino acids of the catalytic triad are indicated by red beads. The amino-terminal region is indicated by  $\text{NH}_2$  at the beginning of the chain, and by  $\text{COOH}$  for the carboxy-terminal region for the end of the chain. The figure was reprinted with permission, from Ref. [29]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The serine residue involved in the catalytic triad typically is a pentapeptide with a conserved consensus sequence (Ser-Gly-X-Gly-X), where X represents any of 20 amino acids. Currently, some databases of conserved domains, for example the PROSITE [43,44] and the Pfam [44] use the sequence of pentapeptide and the region around it to classify a given amino acid sequence encoding a lipase or esterase.

It was observed that all tertiary structures solved with lipases possess the  $\alpha/\beta$  hydrolase configuration [45] (Fig. 1), which has been identified in 1992 by comparing five enzymes with completely different catalytic functions: dieneolactone hydrolase, haloalkane dehalogenase, serine carboxypeptidase II, wheat acetylcholinesterase and *Geotrichum candidum* lipase. These enzymes have no similarity between sequences, do not act on similar substrates, and do not have the same nucleophile donor. However, they have structural similarities, combined with the preservation of the arrangement of the catalytic residues [46]. According to these authors, the number of groups of enzymes with  $\alpha/\beta$  hydrolase configuration has increased, making this one of the most versatile and widely distributed configuration type of proteins.

Besides having the same reaction mechanism, the carboxylesterases and lipases have some structural similarities. The main one is the presence of the  $\alpha/\beta$  hydrolase configuration, consisting of several  $\alpha$ -helices and  $\beta$ -sheets interspersed. This is a fairly common configuration hydrolases, found also in other families of enzymes such as epoxide hydrolases (EC 3.3.2.3) [47] and haloperoxidases (EC 1.11.1.) [48].

#### 4. Lipases role in pathogenicity mechanisms

Pathogenicity has been defined as the ability of a pathogen to infect [49], being their pathogenicity expressed by means of their virulence [50].

The lipase 8 (*LIP8*) gene plays an importance role in the virulence of *Candida albicans*. These researchers observed that Reducing *LIP8* expression found in reduced growth in lipid-containing media and the deficit of mutants in *LIP8* gene were significantly less virulent in a murine intravenous infection model [51]. Also, Feng [52] and Silva [53], reported that Lip5 and Lip8 from *C. albicans* promote host pathogen interaction. Moreover, extracellular phospholipase and proteases enzymes have been pointed as a virulence factor in the pathogenesis of haematogenous infections caused by *C. albicans*, altering their surface characteristics as adherence and penetration [54]. Other studies revealed that some pathogenic microorganisms can grow in the host by using haemoglobin as their source of iron. *Candida* species can produce haemolysins that degrade haemoglobin and extract elemental iron from host cells. Haemolysins have been appointed to be the key of virulence factors because they might promote the survival and persistence of the pathogen in the host [55].

*Candida albicans* is known as an opportunistic pathogen involved in oral, vaginal, and systemic infections and responsible for a high mortality rate in the United States due to their infection in the bloodstream

[56]. This pathogen has been pointed as the most prevalent human fungal pathogen, with an ability to inhabit diverse host niches and cause disease in both immunocompetent and immunocompromised patients [57,58].

*Candida albicans*, in order to exercise its function of infection in the host niches, needs a wide range of virulence factors and fitness attributes [59]. Some of those factors like morphological transition between yeast and hyphal forms, adhesins and invasins, thigmotropism, the formation of biofilms, phenotypic switching, surface hydrophobicity, have been pointed as affecting the *C. albicans* virulence [51,53,60]. Also fitness attributes as rapid adaptation to fluctuations in environmental pH, metabolic flexibility, powerful nutrient acquisition systems and robust stress response machineries have been pointed as virulence factors [59]. Given that, the secretion of aspartic proteinases by *C. albicans* has been reported as the key of their virulence [61–63]. Regarding biofilms production, lipases have a role in helping the pathogen to evade host immune response and the mechanisms of their virulence has been clearly described [64]. According to these researchers, the first step was characterized by the adhesion and colonization of the yeast to the host cell surfaces by the expression of adhesins. Followed by thigmotropism, invasion of host cell through induced endocytosis, biofilm formation, evasion from the host immune response, inflammatory response and finally the host infection; among other factors that influence fungal pathogenicity as i) robust stress response mediated by heat shock proteins, ii) auto-induction of hypha formation iii) excretion of ammonia and concomitant extracellular alkalinisation, etc. Other studies revealed that *C. albicans* can use two mechanisms to invade host cells as induced endocytosis and active penetration [13,65,66]. During the induced endocytosis, the fungus expresses specialized proteins on the cell surface that mediate binding to host ligands thereby triggering the engulfment of the fungal cell into the host cell [14]. The lipases Lip5, Lip6, Lip8 and Lip9 have been reported [67] as some of the secreted by *C. albicans* which enable the organism to adhere and colonize during infection in humans.

Also in mechanisms of infection in plants by fungi, in particular of the genus *Phytophthora* ssp. several authors report the participation of lipases in several steps of these mechanisms [68–73].

One such plant pathogen, *Phytophthora cinnamomi* belongs to the class of Oomycetes, that includes several of the most devastating omnivore pathogens for the natural ecosystems worldwide [52,73,74]. *Phytophthora cinnamomi* is a destructive and widespread soil-borne pathogen which infects woody plant hosts and, in the northeast of Portugal and in the Spanish region, has been reported to affect mainly chestnut-tree (*Castanea sativa*), cherry-tree (*Prunus avium*) and cork oak (*Quercus suber*), [73,75–77]. In fact, *P. cinnamomi*, due to its capacity to destroy natural plant communities and causing diseases with and economically important impact in forestry, horticulture, and in the nursery industry, it's called by some authors as “biological bulldozer” [78].

The infection mechanism of this pathogen has been characterized by

our group in previous studies [75]. Some proteins as endo-1,3-beta-glucanase, endo-glucanase, glucanase inhibitor protein (GIP), necrosis-inducing *Phytophthora* protein 1 (NPP1) and transglutaminase have been pointed to be involved in the mechanisms of infection of this pathogen [79–81]. Pathogenicity of many biotrophic and hemi-biotrophic pathogens has been clearly described [82]. According to these researchers the biotrophic fungi and Oomycetes produce virulence proteins that are recognized and are pathogenic for plants. Their infection process is related to the formation of haustoria within living plant cells. The pathogen penetrates the cell wall and invaginates the plasma membrane during infection, where it forms this specialized feeding structure. Therefore, the haustorium appears to play an essential role in nutrient acquisition and may be involved in the redirection of host metabolism and the suppression of host defences. Among the biotrophic fungi, *Melampsora lini* has yielded the most information regarding the virulence proteins [83] and four proteins (AvrL567, AvrM, AvrP123 and AvrP4) involved in virulence have been reported [84].

In the available nucleotide databases we can find two genomes of *P. cinnamomi* [85,86]. The study of these sequences, their structure functions and interactions will allow elucidating the genetic and molecular mechanisms, defence and attack strategies of the fungus *P. cinnamomi* in order to establish more efficient and resilient control strategies.

In the nucleotide databases <http://www.ebi.ac.uk/ena> and <https://www.ncbi.nlm.nih.gov/>, there are two genome assembly contig set [85] and one transcriptome assembly contig set, from *P. cinnamomi* [86].

The analysis of these sequences has allowed us to identify several ORF's that encode proteins with relevant role in the virulence of *P. cinnamomi*. We have also identified two ORF's that codify proteins with esterase/lipase's domains with similarity to lipases described in other *Phytophthora* species involved in pathogenicity mechanisms. These sequences are in the database “fungidb”, with the references PHYCI\_76143 and PHYCI\_89229 and encode proteins with 309 and 425 amino acids respectively. The primary sequence of these lipases and their alignment by the Clustal algorithm with other lipases of the genus *Phytophthora* is shown in Fig. 2. In addition to the similarity between *Phytophthora* lipases, we can also observe in the figure the common serine residue involved in the catalytic triad with a conserved consensus sequence (Ser-Gly-X-Gly-X), where X represents any of 20 amino acids.

The structural prediction of these *P. cinnamomi* lipases was performed on the Phyre2 server [88], based on the homology of these lipases with the crystallized dog's (*Canis lupus familiaris*) gastric lipase, with access code 1K8Q in Protein Data Bank [89] (Fig. 3).

The extracellular lipases of some pathogens as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes* and *Pseudomonas aeruginosa*, as well as pathogenic fungi, such as *Malassezia furfur* and *Hortaea werneckii*, have been proposed as potential virulence factors [51].

## 5. Biotechnological applications of lipases

Lipases are produced by plants, animals and microbes but only microbial lipases are found to be industrially important with an enormous biotechnological potential, due to the diverse enzymatic properties, substrate specificity, chemoselectivity, regioselectivity and stereoselectivity [9,90]. Even though plant lipases are also available, they are not commercially exploited because of the low yield and the complexity of the processes involved. Regarding the lipases from animal origin, some disadvantages have been reported, among which: the presence of bitter tasting amino acids, the presence of residual animal hormones or viruses and the undesirable effects in the processing of vegetarian diets [11].

Current discoveries about fungal lipases associated with pathogenicity have given these enzymes great versatility with multiple functions and applications.

Esterases and lipases are capable of catalysing a series of reactions, possessing good stability in organic solvents. For that reason, they can be considered excellent biocatalysts in intermediate stages of conventional chemical processes, and in catalysis of chemical reactions involving substrates insoluble in aqueous media. Moreover, they are used in the resolution of racemic mixtures and in the selective removal of certain compounds.

Concerning the microbial lipases, these exhibit a wide range of industrial applications, because of the higher stability, high yield of conversion of substrate into product, the great versatility to adapt to environmental conditions and the simplicity in genetic manipulation and growth conditions [11,90]. A high synthesis rate is obtained with simple processes and low investment and, hence these enzymes are mostly extracellular, their extraction, isolation and purification is simple [91].

The biotechnological potential of lipases is related with their ability to catalyse various reactions. Typically, they preserve the structure and are stable in organic solvents, not requiring the presence of cofactors but requiring stable conditions of temperature and pH. In addition, these enzymes exhibit a broad substrate specificity and high enantioselectivity [24,91–94].

Nowadays, lipases are used for the generation of enantiomerically enriched primary and secondary alcohols and, to a lesser extent, to obtaining chiral carboxylic acids and secondary amines [15]. The stereoselectivity of lipases is useful in the synthesis of biopolymers such as, for example, polyesters and polyphenols in the kinetic resolution of racemic mixtures in the secondary hydrolysis reactions, the transesterification and alcohol etherification [16,25].

The panorama of lipases use encompasses many industries: enzymes of microbial origin are used in food industry, in the manufacture of detergents (hydrolysis of fats), cosmetics (removal of lipids) and wastewater treatment (decomposition and removal of oily substances). As these enzymes have enormous catalytic potential, they are also used as ideal biocatalysts in organic chemistry, fine chemicals (synthetic esters) in the pharmaceutical industry, and in the production of food additives (flavour enhancement) [11,91,93].

### 5.1. Pharmaceutical industry

The synthesis of bioactive substances has been practiced over the years by means of conventional organic chemistry. This synthesis route, in some cases, can result in problems such as instability of the molecule on the reaction conditions and the formation of racemic mixture [26]. The ability of lipases to resolve racemic mixtures by the synthesis of a single enantiomer is currently exploited for drug production by the pharmaceutical industry.

Microbial lipases are used to concentrate polyunsaturated fatty acids from animal and botanical lipids, and their mono and diacylglycerides are used to produce a variety of pharmaceuticals. The use of lipases in pharmaceutical industry is due, in particular, to the enantioselectivity exhibited by many of these enzymes. The enantioselective catalysis allows the obtention of optically pure products, since these enzymes are chiral molecules capable of recognizing and acting preferably in the isomers of a racemic mixture. This feature is extremely advantageous because, in many cases, the isomers (R and S) have a biological activity, while others are less active or even toxic [20].

One important compound used in the synthesis of non-steroids anti-inflammatory drugs (Ibuprofen and Naxopreno) is 2-phenyl-propanoic acid, which can be obtained by transesterification reactions or hydrolysis of the corresponding ester catalysed by lipases [19]. Another example is the use of a *Candida antarctica* lipase resolution of racemic thiotetronic acid derivatives to obtain the compound (R)-thiolactomycin which has a chiral quaternary carbon atom C5 [15,19,95].

Lipases are also used in the production of antidepressants, anti-hypertensives and vasodilators [18].





**Fig. 2.** Alignment of the primary structures of *P. cinnamomi* (PHYCI\_110663 and PHYCI\_89229), *P. infestans* (PITG\_01274), and *P. ramorum* (PSURA\_76385) lipases (FungiDB). The rectangle delimits a conserved consensus sequence (Ser-Gly-X-Gly-X) the serine residue involved in the catalytic triad and under the yellow star the active site of a lipase contains a catalytic triad consisting of Ser - His - Glu. The software Clustal Omega multiple sequence alignment program, was used [87]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

## 5.2. Food industry

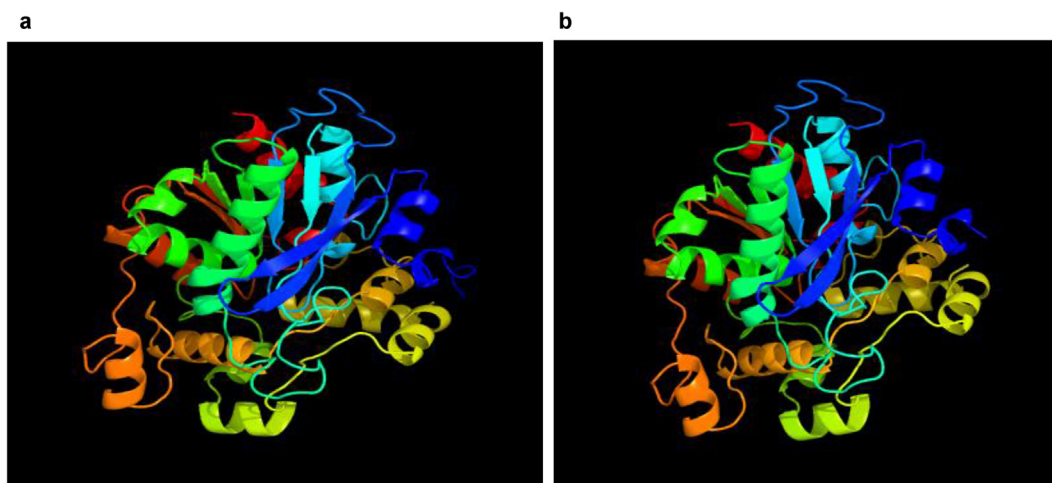
These enzymes have been extensively used in the food industry, especially in the dairy industry for the hydrolysis of milk fat, the modification of the fatty acid chain lengths to enhance the cheese characteristics and, more recently, to accelerate cheese ripening and the lipolysis of butter fat and cream. In addition, lipases have been used in selective hydrolysis of fat, allowing its use in the formation of flavoured products, in the production of substitutes for butter and other additives used in cereals, snacks and chewing. The addition of these hydrolysates provides a variety of food sensory characteristics [96]. Lipases are also used to modify the taste of food, the synthesis of esters of fatty acids, and short chain alcohols, these being the basic flavour and aroma compounds [20].

Another example can be given in the baking industry, in the manufacture of bread lipase degrade lipids from wheat changing its

interaction with the gluten, obtaining a dough conditioner result in increasing bread volume and improved texture. Hydrolysis performed by specific lipases is applied to obtain monoacylglycerol which are used as emulsifying agents [97].

## 5.3. Detergent industry

The use of enzymes in detergent formulations is common in developed countries, with over half of all detergents presently available containing enzymes. Lipases are used in the detergent industry to facilitate the breaking of bonds present in the triglycerides and hence solubilise grease adhered to the fabric, therefore enhancing the ability to remove tough stains. These enzymes make the detergent environmentally safe, by reducing the environmental load, as they save energy by enabling a lower wash temperature. Also, the products are mostly biodegradable and leave no harmful residues [5].



**Fig. 3.** 3-D structure prediction of *P. cinnamomi* lipases, based on template model dog gastric lipase (1K8Q in PDB) with 371 residues for PHYCI\_110663 (left figure a) and 367 residues for PHYCI\_89229 (right figure b) and 86% modelled with 100.0% confidence by the single highest scoring template. The structural predictions were performed through the Phyre2 server (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>) and the structural representation was performed by Pymol program, who's the version can be installed in <https://pymol.org/2/>.

Examples of enzymes used in detergents are Lipolase® (Novozymes) obtained from the fungus *Thermomyces lanuginosa* and expressed in *Aspergillus oryzae*; the Lumafast® (Genencor, USA) and Lipomax® (Gist-Brocades, The Netherlands), bacterial lipases from *Pseudomonas mendocina*, and *Pseudomonas alcaligenes* [27].

The most important commercial application area for hydrolytic lipases is industrial or household cleaners [23], which is generally used in combination with one or more enzymes such as proteases, amylases and cellulases, responsible for the removal of various fats [18].

### Final remarks

The recent DNA sequencing techniques with platforms that allow massive sequencing, combined with advanced bioinformatics capabilities allow us to deduce the role of many molecular factors in metabolic pathways. It is possible that many molecules have roles in metabolic pathways that until our days are unknown. The recent associations of lipases in the pathogenicity mechanisms of some microorganisms confirms the high versatility of these enzymes. However, much is unknown about the concrete action of these enzymes in the metabolic pathways involved in pathogenicity mechanisms. We think that the silencing of lipase genes in phytopathogenic fungi and subsequent plant infection with these fungi may help to better understand the role of these enzymes in the infection.

The discovery of molecule factors and their interactions in the metabolic pathways of infection is an important step in the development of prevention strategies, therapy processes and control of fungal infections in plants.

### Conflicts of interest

The authors declare that there are no conflicts of interest. Also, are indebted to the careful and constructive criticisms of the reviewers.

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