

Microbial lipases: propitious biocatalysts for various biotechnological applications

Lipases microbianas: biocatalisadores propícios para diversas aplicações biotecnológicas

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Abstract

The use of microbial lipases is very important because they have a broad spectrum of catalytic reactions. Lipases catalyze reactions in aqueous and non-aqueous media and have advantages when compared to chemical catalysts, due to their specificity, enantioselectivity and stability at pH and temperature. The interest in research for microbial lipases occurs because of their applicability in various sectors and industries, such as food and beverage production, cosmetics, pharmaceuticals, surfactants, dairy products, treatment of effluents containing oils and fats, biofuels, among others. Biocatalysts can be obtained by submerged fermentation (SF) or solid-state fermentation (SSF), which has advantages over SF, because it is possible to use agroindustrial waste as substrate or growth support for microorganisms, becoming an alternative to reduce production costs. SSF is a promising technology for enzyme production, because besides using low-cost substrates, the biocatalyst can be produced in a more concentrated form, facilitating its recovery from the culture medium when necessary. Thus, this paper intends to discuss and review studies on microbial lipases, with direct application of the fermented solid (SSF), focusing on the main microorganisms, substrates and supports used in SSF, applicability of lipases in several industrial sectors, besides presenting conversion results

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using different microorganisms or/and substrates.

Keywords: Enzyme; Lipase; Biocatalyst; Catalytic activity; Biotechnology.

Resumo

O uso de lipases microbianas é de extrema importância, pois possuem um amplo espectro de reações catalíticas. As lipases catalisam reações em meios aquosos e não aquosos e apresentam vantagens quando comparadas aos catalisadores químicos, devido à sua especificidade, enantiosseletividade e estabilidade em pH e temperatura. O interesse na pesquisa de lipases microbianas ocorre devido à sua aplicabilidade em diversos setores e indústrias, como produção de alimentos e bebidas, cosméticos, farmacêuticos, surfactantes, laticínios, tratamento de efluentes contendo óleos e gorduras, biocombustíveis, entre outros. Os biocatalisadores podem ser obtidos por fermentação submersa (FS) ou fermentação em estado sólido (FES), o que apresenta vantagens em relação à SF, pois é possível utilizar resíduos agroindustriais como substrato ou suporte de crescimento de microrganismos, tornando-se uma alternativa para reduzir custos de produção. O SSF é uma tecnologia promissora para produção de enzimas, pois além de utilizar substratos de baixo custo, o biocatalisador pode ser produzido de forma mais concentrada, facilitando sua recuperação do meio de cultura quando necessário. Assim, este artigo pretende discutir e revisar estudos sobre lipases microbianas, com aplicação direta do sólido fermentado (SSF), focando nos principais microrganismos, substratos e suportes utilizados em SSF, aplicabilidade de lipases em diversos setores industriais, além de apresentar resultados de conversão usando diferentes microrganismos e/ou substratos.

Palavras-chave: Enzima; Biocatalisador; Lipase; Atividade catalítica; Biotecnologia.

1 Introduction

Enzymes are biological catalysts that accelerate the speed of chemical reactions and metabolic processes, have high specificity and are highlights in the area of bioprocess technology (TAHERI-KAFRANI *et al.*, 2021). Most enzymes are proteins, but some are considered ribonucleic acids and classified according to their catalysis reactions as: oxidoreductases (oxide reduction), transferases (transfer of groups of molecules), hydrolases (hydrolysis), lyases (bond breaking), isomerases (intramolecular change), Translocases (assists in moving another molecule) and ligases (formation of a covalent bond between two molecules with high energy consumption) (CAI; CHOU, 2005; TAO *et al.*, 2020).

The use of biological catalysts (enzymes) has become a viable option and the interest in this area is growing more and more due to their physicochemical characteristics (THAPA *et al.*, 2019), with higher biodegradability, application in milder conditions, high recoverability in the process (CZAJA, 2015; GRAJALES-HERNÁNDEZ *et al.*, 2020; SHAKERIAN; ZHAO; LI, 2020), in addition to their ability of chemoselectivity, enantioselectivity and regioselectivity (TONG; BUSK; LANGE, 2015; KRETSCHMANN *et al.*, 2019; GIRELLI; ASTOLFI; SCUTO, 2019).

There is a growing search for viable alternatives for obtaining enzymes, especially those derived from microorganisms, as they tend to be environmentally friendly and economically relevant when compared to enzymes of animal and plant origin (VYAS; CHHABRA, 2017; CARVALHO *et al.*, 2021). Moreover, microorganisms are the main sources of enzymes due to easy procurement, wide availability, simple nutritional requirements, rapid growth rate, and diversity in catalytic activities (THAPA *et al.*, 2019).

Among the enzyme groups, hydrolases are widely used in the industrial field (AMADI *et al.*, 2020), and lipolytic enzymes (belonging to hydrolases) are the third most widely used group of catalysts and considered the most important biocatalyst in the field of biotechnology (KUMAR *et al.*, 2020). The high versatility of lipases allow applications in various sectors such as food industry (ZHANG *et al.*, 2018), pharmaceutical (NAGHDI *et al.*, 2018), textile, pulp and paper, dairy, detergent (and other cleaning products) manufacturing, new polymeric materials, wastewater treatment and biofuels (BHARATHI; RAJALAKSHMI, 2019; KUMAR *et al.*, 2020).

In view of this, studies are emerging with new supports to immobilize the enzymes, such as agroindustrial waste, which can be more viable alternatives of supports for enzyme immobilization, because besides being a more economical way to make the process viable, it will also help in the problems of disposal and disposal of waste, following the principles of green chemistry.

Thus, this paper aims to discuss and review studies on microbial lipases, with direct application of the fermented solid, focusing on the main microorganisms, substrates and supports used in SSF and applicability of lipases in various industrial sectors.

2 Theoretical reference

2.1 Lipase structures and characteristics

Lipases (EC 3.1.1.3) also known as triacylglycerol ester hydrolases are abundant in nature and belong to the group of enzymes of the serine hydrolase category (BHARATHI; RAJALAKSHMI; KOMATHI, 2018). Their molecular weight is in the range of 19 kDa to 60 kDa besides being described as monomeric protein (formed by only one polypeptide chain) (CHANDRA; SINGH; ARORA, 2020), and what differentiates lipases from other types of esterases is the ability to hydrolyze water insoluble esters (KARTAL, 2016).

The essential structural elements of lipases are: binding pocket, cap, oxy-anion hole and disulfide bridge. The binding pocket is hydrophobic in nature and is related to enzyme activity (FARYAD *et al.*, 2021). The cap is important for determining molecular activity and selectivity in addition to interfacial activation (KHAN *et al.*, 2017), the oxy-anion hole is of utmost importance as it is this region that can influence the catalytic efficiency of lipases (GAO *et al.*, 2011) and the disulfide bridge contributes to conformational stability, decreasing entropy and influencing the thermal stability of the enzyme (TAMBUNAN; RANDY; PARIKESIT, 2014).

Lipases have the characteristic of increasing catalytic activity at water-oil interface, being a process known as interfacial activation, involving a structural rearrangement of conformation from inactive to active (KHAN *et al.*, 2017). With this, catalysis is initiated by anchoring the lipase at the oil-water interface through the exposed hydrophobic area (PRIYANKA *et al.*, 2018).

Thus, lipases can catalyze hydrolysis and synthesis of triglycerides, diacylglycerol, monoacylglycerol, and glycerol, and also exhibit hydrolysis, interesterification, esterification, aminolysis, acidolysis, and alcoholysis activities (JAVED *et al.*, 2018). In addition to the aforementioned potential, lipases synthesize glycerol esters as well as long-chain fatty acids in non-aqueous medium, contributing in a wide range of industrial applications (CHANDRA; SINGH; ARORA, 2020).

Also at oil-water interface, lipase has the ability to hydrolyze triglycerides, thus converting them into glycerol and fatty acids, and has properties that reverse this reaction in aqueous and non-aqueous media (LEE *et al.*, 2015; RAMOS-SÁNCHEZ *et al.*, 2015). The efficiency of lipases depends on the physical properties and factors such as position of the fatty acid in the glycerol structure, chain length and degree of unsaturation (TONG; BUSK; LANGE, 2015; BONOMI; IAMETTI; MARENGO, 2019).

Given the advantageous characteristics that lipases have as catalysts, these enzymes are in third place in industrial application, behind proteases and amylases (carbohydrases), this is

due to their great versatility (JAVED *et al.*, 2018; ARORA, MISHRA; MISHRA, 2020). The efficiency of the application of lipases will also depend on the origin, being microbial (bacteria, fungi and yeast) the most used (VYAS; CHHABRA, 2017).

2.2 Lipases of bacterial origin

In the industrial arena, lipases can come from bacteria, which in most cases are considered safe and do not produce toxins (NIYONZIMA; MORE, 2014). Bacterial lipases are less diverse compared to fungal lipases and are more limited in industry, however they are still in high demand due to their yield and ability to work in alkaline pH (BHARATHI; RAJALAKSHMI; KOMATHI, 2018).

In most bacteria, the lipases produced by them are affected by polysaccharides found in the medium, and usually these lipases are glycoproteins, but in some cases of extracellular lipases their nature is considered lipoproteinic (CHANDRA; SINGH; ARORA, 2020). Extracellular lipases coming from bacteria will depend on some nutritional and physicochemical factors such as: carbon and nitrogen sources, oxygen, lipids, incubation time, pH, among others (ARORA; MISHRA; MISHRA, 2020).

Among the genera with the ability to produce lipases, the best known are *Bacillus*, *Burkholderia*, *Pseudomonas* and *Staphylococcus* (BOUAZIZ *et al.*, 2011; SAGAR *et al.*, 2013; ARORA; MISHRA; MISHRA, 2020). In addition to these genera, others such as *Achromobacter* spp., *Alcaligenes* sp., *Arthrobacter* spp., and *Chromobacterium* spp. (CHANDRA; SINGH; ARORA, 2020). Table 1, we can see some species of lipase producing bacteria and their respective applications in industry.

Table 1. Bacterial species that produce lipases and their applications in biotechnology

Bacterial species	Applications	References
<i>Achromobacter</i> sp.	Oily wastewater treatment.	(DENG <i>et al.</i> , 2020).
<i>Bacillus aerius</i>	Production of biodiesel.	(BHAN; SINGH, 2020).
<i>Bacillus cereus</i>	Wastewater treatment.	(DURVAL <i>et al.</i> , 2020).
<i>Bacillus</i> sp.	Food industry.	(BALAJI; CHITTOOR; JAYARAMAN, 2020).
<i>Bacillus subtilis</i> and <i>Bacillus thermocatenulatus</i>	Medical Industry.	(SU; FANG; ZHANG, 2020).
<i>Burkholderia cepacia</i>	Production of biodiesel.	(OSTOJČIĆ <i>et al.</i> , 2020).
<i>Geobacillus stearothermophilus</i>	Detergent formulation.	(ABOL-FOTOUH; ALHAGAR; HASSAN, 2021).
<i>Lactobacillus casei</i>	Cheese industry (flavorings).	(DE SOUZA; RIBEIRO; COELHO, 2019).

<i>Pseudomonas cepacia</i> and <i>Pseudomonas</i> sp.	Production of biodiesel.	(KUMAR <i>et al.</i> , 2017).
<i>Serratia marcescens</i>	Manufacture of detergent and biodiesel.	(GARCÍA-SILVERA <i>et al.</i> , 2018).
<i>Staphylococcus aureus</i>	Detergent industry.	(BACHA <i>et al.</i> , 2018).

Within the family *Bacillaceae*, bacteria belonging to the genera *Bacillus* and *Geobacillus* are the main sources of lipase production. Bacteria of the genus *Bacillus* have great potential for biotechnological applications due to their properties, such as cells that adapt to survive in extreme climatic conditions (GUNCHEVA; ZHIRYAKOVA, 2011).

Lipase from *Bacillus* species occurs by SF (CHAKRABORTY; RAJ, 2008; BEREKAA *et al.*, 2019). Its cells are grown in a nutrient medium rich in carbon, nitrogen, phosphorus and mineral salts (Ebrahimpour *et al.*, 2008), as well as lipids (olive, mustard, soybean, rice bran, cottonseed, sesame and corn oil) and free fatty acids (oleic acid) (GUNCHEVA; ZHIRYAKOVA, 2011).

2.3 Lipase from yeast and filamentous fungi

Lipases from yeast are widely used in the industrial arena as they are considered safe and non-toxic (MELANI; TAMBOURGI; SILVEIRA, 2020). Moreover, they have unique applications in the chemical and pharmaceutical sectors, biodiesel production, and the food industry (SINGH; MUKHOPADHYAY, 2012).

Most yeasts possess the ability to produce lipases (ALAMI *et al.*, 2017), but the main producing genera are *Candida*, *Rhodotorula*, *Yarrowia*, and *Trichosporon*. In addition to the above, other yeast genera are considered good lipase producers such as *Saccharomyces*, *Torulospora*, *Kluyveromyces*, *Pseudozyma*, *Pischia*, *Lachancea*, and *Zygosaccharomyces* (MOFTAH *et al.*, 2012; NAGARAJAN, 2012; DIVYA; PADMA, 2015; LAN *et al.*, 2016; SU; FANG; ZHANG, 2020).

Yeasts of the genus *Candida* are highlighted with great industrial potential for the production of extracellular lipase and have as an advantage the ability to act in long-chain ester, hydrolyzing and synthesizing with this, various types of oils (ALAMI *et al.*, 2017). Within this genus there are species considered excellent lipase producers and with greater potential within the category of yeasts, containing several reports in the literature on their properties, structures and catalytic actions (BHARATHI; RAJALAKSHMI, 2019).

The *Candida* genera also possess the ability to hydrolyze nonspecific triacylglycerols that are found abundantly in nature. With this, several species of this genus are widely studied

for their ability to produce extracellular lipase such as *C. albicans*, *C. antarctica*, *C. deformans* CBS 2071, *C. curvata*, *C. rugosa*, *C. albidus*, *C. laurentii*, *C. zeylanoides*, *C. famata*, *C. lipolytica* (ALAMI *et al.*, 2017).

Several species of yeast and filamentous fungi are good lipase producers with simple extraction, purification and processing steps (ROY *et al.*, 2021). Filamentous fungi have as a characteristic, the ability to produce extracellular enzymes, thus making them an attractive source for enzymes of industrial interest such as lipases (GUTARRA *et al.*, 2009).

Genera of filamentous fungi such as *Penicillium*, *Rhizopus*, *Aspergillus*, *Fusarium*, *Mucor* and *Geotrichum* are examples of lipase producers with potential application in various industrial areas (MAHMOUD *et al.*, 2015; ÇAKMAK; AYDOĞDU, 2021). In Table 2 we can observe some yeast and filamentous fungi species and their applicability in the industrial field.

Table 2. Yeast and filamentous fungi species that produce lipases and their applicability in biotechnology

Yeast and filamentous fungi species	Applications	References
<i>Candida rugosa</i>	Synthesis of flavor and aroma esters, biodiesel production, and other chemical producer syntheses.	(BAYRAMOGLU <i>et al.</i> , 2022; SUBROTO <i>et al.</i> , 2020).
<i>Candida antarctica</i>	Production of biodiesel, detergent, food, chemical industry, among others.	(SHAHEDI <i>et al.</i> , 2019; MONTEIRO <i>et al.</i> , 2021).
<i>Yarrowia lipolytica</i>	Flavoring esters, wax esters biolubricants, food industry, biosensors, biodiesel.	(DE SOUZA; RIBEIRO; COELHO, 2019; MADZAK, 2018).
<i>Rhodotorula</i> sp.	Formulation of detergents.	(MAHARANA; SINGH, 2018).
<i>Trichosporon</i> sp.	Synthesis of structured triacylglycerols, surfactants, food additives, biofuels, lubricants and detergents.	(CAO <i>et al.</i> , 2021).
<i>Saccharomyces cerevisiae</i>	Bioremediation.	(MASSOUD <i>et al.</i> , 2019).
<i>Aspergillus niger</i>	Food industry and biodiesel production.	(FENG <i>et al.</i> , 2020).
<i>Aspergillus nidulans</i> and <i>Aspergillus oryzae</i>	Biofuel production and Bioremediation.	(KUMAR <i>et al.</i> , 2020; OSTOJČIĆ <i>et al.</i> , 2020).
<i>Penicillium</i> sp.	Bioremediation and Biodegradation.	(BARNES <i>et al.</i> , 2018).

<i>Penicillium roqueforti</i> and <i>Penicillium camemberti</i>	Cheese manufacturing.	(KUMURA <i>et al.</i> , 2019).
<i>Geotrichum candidum</i>	Chemical industry.	(BRABCOVÁ <i>et al.</i> , 2013).
<i>Rhizopus oryzae</i>	Production of structured lipids, biodiesel and flavor esters.	(LÓPEZ-FERNANDEZ; BENAIGES; VALERO, 2020).
<i>Fusarium</i> spp.	Chemical industry.	(RANA <i>et al.</i> , 2019).
<i>Fusarium incarnatum</i>	Bioremediation.	(JOSHI; SHARMA; KUILA, 2019).
<i>Mucor miehei</i>	Production of biodiesel.	(CARTERET; JACOBY; BLIN, 2018).

Filamentous fungi vary in their lipolytic production depending on the strain, presence of inducer, carbon and nitrogen sources, pH, NaCl concentration, temperature, among other medium conditions that make it indispensable in obtaining the best yield of extracellular enzymes (WADIA; JAIN, 2017). Fungal lipase production can occur by SF or SSF, but filamentous fungi are more adapted with growth in solid-state fermentation than other microorganisms such as bacteria and yeast (EDWINOLIVER *et al.*, 2010).

2.4 Analysis of fermentation techniques for lipase production

The fermentative processes for lipase production (SF and SSF) are widely used in obtaining products, mainly from the industrial sector, this being an approach that uses microorganisms for biological transformations of complex substrates into simpler molecules (BHARATHI; RAJALAKSHMI, 2019). Both are conventional techniques used for lipase production and each of them have their advantages and peculiarities (FARYAD *et al.*, 2021).

2.5 Submerged Fermentation (SF)

In the process of SF, microorganisms are found in normally liquid media, presenting greater homogeneity in the culture medium and parameters such as temperature and pH are easily controlled and the concentration of dissolved nutrients are well defined. The extracellular production of SF is based on the optimization of carbon source, nitrogen, oils and surfactants, in addition to physicochemical parameters such as pH and temperature and incubation time (GEOFFRY; ACHUR, 2018).

Lipases from SF are generally more thermostable than those obtained by SSF process, making them suitable for industrial applications involving high temperatures, in addition, lipase production by SF generates less undesirable metabolites such as phenolic compounds, phenolic acids and flavonoids than SSF (COLLA *et al.*, 2015). Thus, SF is the most widely used method for obtaining enzymes of industrial interest because it has advantages such as easy large-scale

obtainability (ALABDALALL *et al.*, 2020). In addition, lipase from SF can be subjected to drying techniques to obtain dry extract of the enzyme, making it more durable and increasing its shelf life (UTAMI *et al.*, 2017).

2.6 Solid-State Fermentation (SSF)

Solid-state fermentation involves the use of a solid matrix and the process is carried out in the absence or near absence of free water, so for microorganisms to thrive, the substrate or support must contain moisture and provide the nutrients necessary to keep their metabolism active and provide for their growth (VANDENBERGHE *et al.*, 2021).

Agricultural and forestry residue substrates such as cereal grains, legume seeds, bran such as oatmeal and soybean meal, cakes (press cake or oil cake are the solid residues obtained after processing oilseeds), sugarcane and cassava bagasse, fruit and coffee pulp and peel, straws, sawdust, wood chips, materials of plant and animal origin, are the most commonly used in the process of SSF and most of them have low cost, are easily obtained and provide all necessary nutrients for the growth of microorganisms (FARINAS, 2015; SADH; DUHAN; DUHAN, 2018; PALUZAR; TUNCAY; AYDOGDU, 2021; DE MOURA-DICKEL *et al.*, 2022).

This type of fermentation can be considered an economical way, using relatively simple substrates in the production of extracellular enzymes, mainly coming from filamentous fungi by providing a similarity to their natural habitat, in which microorganisms will grow and release in their metabolism, products with high added value (STEUDLER; WERNER; WALTHER, 2019).

The main advantages of using SSF are lower susceptibility to contamination, lower sterilization requirements, higher enzyme productivity, lower susceptibility to substrate inhibition, use of agro-industrial wastes (such as those mentioned above), lower effluent production, higher substrate quality and activity without the need for the addition of organic solvents, making the process more environmentally friendly and economical (SOCCOL *et al.*, 2017).

In contrast, we can cite negative points regarding the use of SSF, such as a limited number of species that are able to thrive in environments with reduced humidity (FARYAD *et al.*, 2021).

When considering the wide applicability of lipase and the few areas covered by SSF lipases, we can observe the need for further exploration in this area (AGUIEIRAS *et al.*, 2018). In Table

3 we can observe some works using fermented solids and their applications

Table 3. Fermented solid lipases from microorganisms and their applications in biotechnology

Species of microorganisms	Fermented solid	Applications	References
<i>Aspergillus ibericus</i>	Palm kernel and sesame oil pie mixes	Flavor Esters	(OLIVEIRA <i>et al.</i> , 2017).
<i>Aspergillus ibericus</i>	palm kernel oil pie and other types of oil pies	Flavor Ester Production	(OLIVEIRA <i>et al.</i> , 2017).
<i>Aspergillus niger</i>	Wheat bran / Corn cob	Bioremediation	(KRELING <i>et al.</i> , 2020).
<i>Aspergillus niger</i>	Canola Pie	Cooking oil waste treatment	(PRECZESKI <i>et al.</i> , 2018).
<i>Aspergillus niger</i>	Copra residue (dried coconut pulp)	Wastewater treatment	(ZULKIFLI; RASIT, 2020).
<i>Aspergillus niger</i> / <i>Penicillium</i> sp.	Orange residue	Medications	(ATHANÁZIO-HELIODORO <i>et al.</i> , 2018).
<i>Penicillium sumatrense</i> / <i>Aspergillus fumigatus</i>	Sunflower Seed	Methyl Oleate	(OLIVEIRA <i>et al.</i> , 2020).
<i>Anoxybacillus</i> sp.	Mustard Pie	Detergent Manufacturing	(SAHOO <i>et al.</i> , 2020).
<i>Yarrowia lipolytica</i>	Watermelon rinds	Depolymerization of polyethylene terephthalate (PET)	SALES <i>et al.</i> , 2020
<i>Candida viswanathii</i>	Wheat bran with barley grain	Chicken Fat Hydrolysis	ALMEIDA <i>et al.</i> , 2016

3 Conclusions

Lipases are enzymes with an important enzyme and are capable of catalyzing various reactions, which makes them very useful biocatalysts for industrial applications. Agro-industrial wastes are used to solid state fermentation. The lipases immobilized in fermented solids, coming from microorganisms, allow a wide applicability covering several industrial sectors, being this, a way to solve the high cost with immobilization, leaving them economically viable. The use of lipase in industrial processes addresses the concept of green chemistry, because it reduces unwanted products, presents good yield during the process, generates biodegradable products, is environmentally safe and has high recovery capacity after the

process, thus contributing to the reduction of environmental impacts. With this, we observe that several microorganisms have already proven effective in the various industrial applications using agroindustrial residues rich in fatty acids, easy to obtain and with a wide variety of options, which can be adapted to each region, making the production process more viable.

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