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Review

Staphylococcal lipases: Biotechnological applications

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ABSTRACT

The production of extracellular lipases by staphylococcal species has been known for many years [1,2]. Interest in these lipases was originally stimulated by observations that certain pathogenic staphylococci possess lipolytic activity. There are several indications that they are involved in the release of free fatty acids in blood plasma and in skin colonization and related diseases [3,4]. In addition to this physiological importance, a research was stimulated by the potential use of staphylococcal lipases to synthesize many molecules with high value added. Recently, different staphylococcal lipases were isolated, purified and biochemically characterized. An increased interest of these lipases results from their potential in modern biotechnology. These new lipases are immobilized to be used in non aqueous media as biocatalyst to catalyze the transesterification, the alcoholysis and the esterification of the alcohols with organic acids. This review describes various applications of staphylococcal lipases in detergent, food, flavor, biopolymers, esters and antioxidant.

Because of their biological significance and their increasing importance in biotechnology, a thorough understanding of staphylococcal lipases functioning is needed.

Contents

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1. Introduction

Lipases (EC 3.1.1.3) are an important group of biotechnologically valuable enzymes which are widely distributed in nature [5]. Although lipases have been found in many species of animals, plants, bacteria, yeast and fungi, the microbial lipases are the most interesting because of their potential applications in various industries such as food, dairy, pharmaceutical, detergents, textile, biodiesel and cosmetic industries and in synthesis of fine chemicals, agrochemicals and new polymeric materials [6,7]. Bacterial lipases received much attention for their substrate specificity and their ability to function in extreme environments. The high-level production of these enzymes requires not only the efficient overexpression of the corresponding genes but also a detailed understanding of the molecular mechanisms governing their folding and secretion [7,8].

Many microorganisms produce lipases which are secreted into growth medium. A part from their biological significance in processes such as skin colonization and pathogenesis[3,4], an increased interest in microbial lipases results from their potential in modern biotechnology. Some of the industrially important chemicals manufactured from fats and oils by chemical processes could be produced by lipases under mild conditions with greater rapidity and better specificity [9,10]. The stereo-, regio- and enantiospecific behaviors of these enzymes have caused tremendous interest among scientists and industrialists [11]. Because of their broad substrate selectivity, their enantioselectivities and their stability at high temperature and in organic solvents, microbial lipases are frequently used as biocatalysts, for example in the production of fine chemicals and as additives in detergents.

The production of extracellular lipases by staphylococcal species has been known for many years [1,2]. Interest in these enzymes was originally stimulated by their ability to catalyze the esterification, interesterification, and transesterification in non-aqueous media. In the past years, several staphylococcal lipases were purified and biochemically characterized [12–15]. Several years ago, our interest became focused on lipases from staphylococcal species.

Recently, our knowledge on staphylococcal lipases has been improved considerably by the screening of new lipases having a wide range of properties which are suitable for many applications [16–19]. Furthermore, novel biotechnological applications have been successfully established using staphylococcal lipases for the synthesis of biopolymers and biodiesel, the production of antioxidants and flavor compounds [20,22].

The present review will concentrate on the lipases produced by the members of the Gram-positive genus *Staphylococcus* and will focus on their biotechnological applications.

2. Characterization of staphylococcal lipases

Lipolytic activity in staphylococci had already been described as early as 1901 by Eijkmann [23]. This activity is responsible for the release of considerable amounts of fatty acids, particularly octadecenoic acid, by *Staphylococcus aureus* in human plasma [24]. In earlier times, various staphylococcal lipases were purified and some biochemical properties were investigated [25–27]. Their corresponding genes have been identified. It appears that the mature forms of these lipases are very homologous, and share 50–99% amino acid sequence identity. Despite the high similarity of these enzymes, important differences in biochemical properties, pH profile, temperature and pH stability, substrate specificity and chain length selectivity have been described (Table 1).

The biochemical properties showed that SAL1 and SEL are closely related. They are active over a broad pH range, with an optimum around pH 6 [14,29]. Accordingly, both lipases are stable under acidic conditions, whereas they are inactivated at pH values above 10. This preference of acidic conditions is quite unusual among bacterial lipases, which in most cases exert their highest activities at alkaline pH. For SHyL, SSL, SXL1 and SXL2, the pH optimum was 8.5 [14,16,17,19]. Unlike all previously described staphylococcal lipases, the SAL3 is found to be active and stable at alkaline conditions [18]. The SAL3 was fully active at pH range of 8–10 and remains stable at abroad range of pH values between 5 and 12 after 24 h of incubation time.

The differences in pH dependency of SAL-1, SEL-3, SHyL, SSL, SXL1, SXL2 and SAL3 are also reflected in their substrate preferences. SAL-1 and SEL-3 exhibit a strong preference for glycerides with short-chain fatty acids [14,29]. Both lipases have a significant bias towards substrate molecules with butyric acid esterified to glycerol, p-nitrophenol, or umbelliferone. Corresponding ester compounds with an acyl chain length of one methyl group above or below this size, e.g., triacetylglycerol or tripentanoylglycerol, are poorly hydrolyzed by these enzymes [14,29]. A similar chain-length preference has been found for SHaL, SWL, SXL2 and SAL3, which also exhibit the highest lipolytic activities with tributyrylglycerol [18,19,26,29,30]. In contrast, SHyL [14], SSL [16] and SXL1 [17] hydrolyze triacylglycerols or p-nitrophenyl esters almost irrespective of their chain length. The SHyL differs from all these lipases in being very tolerant towards lipid compounds with different chain lengths. Furthermore, SHyL is distinguished in that it readily recognizes phospholipids of different chain lengths as substrates and thus can also be regarded as a phospholipase [31]. To date, SHyL is unique among wild type staphylococcal lipases in having a very broad substrate spectrum ranging from lipids of various chain lengths to phospholipids and lysophospholipids [14]. Recently, we have shown that the mutant G311L SXL1 displays a novel phospholipase activity. This newly construct is able to hydrolyze not

Table 1
Biochemical properties of different staphylococcal lipases.

Strain	Mw (kDa)	Optimum temperature (°C)	pH optimum	Specific activity (U/mg)	References
Staphylococcus aureus NCTC 8530 (SAL1)	45	37	6	636	[14,28]
Staphylococcus hyicus DSM20459 (SHyL)	45	37	8.5	–	[27]
Staphylococcus epidermidis RP62A (SEL3)	45	37	6	–	[14,27]
Staphylococcus warneri 863 (SWL)	45	25	9	1860	[26]
Staphylococcus simulans (SSL)	45	37 ($t_{1/2}$ = 5 min at 60 °C)	8.5	1000 (TC_4) 1000 (O.O)	[16]
Staphylococcus xylosus (*SXL1*)	43	45 ($t_{1/2}$ = 15 min at 60 °C)	8.2 (5–8.5)	1900 (TC_4)19 1900 (O.O)	[17]
Staphylococcus aureus (*SAL3*)	45	55 ($t_{1/2}$ = 60 min at 60 °C)	9.5 (5–12)	4200 (TC_4) 3500 (O.O)	[18]
Staphylococcus xylosus (*SXL3*)	43	45 ($t_{1/2}$ = 15 min at 95 °C)	9	10.15 (*p*-nitrophenyl acetate)	[41]
Staphylococcus xylosus (*SXL2*)	43	55 ($t_{1/2}$ = 60 min at 57 °C)	8.5 (4–11)	6300 (TC_4) 2850 (O.O)	[19]

only one but two ester bonds at the sn-1 and sn-2 positions of phospholipids [32].

3. Application of staphylococcal lipases

3.1. Importance of thermostable and alcaline staphylococcal lipases

Thermostable lipases from microbial sources are highly advantageous, for biotechnological applications, since they can be produced at low cost and exhibit improved stability [33]. In recent years there has been a great demand for thermostable lipases in industrial fields. The importance of alkaline and thermostable lipases for different applications has been grown rapidly. A great deal of research is currently going into developing lipases, which will work under alkaline conditions as fat stain removers. Thus thermostable lipases from various sources have been purified and characterized [34–39]. These enzymes can be used to the synthesis of biopolymers and biodiesel and used for the production of pharmaceuticals, agrochemicals, cosmetics, and flavors [40].

Recently, some thermostable lipases from *Staphylococcus* sp. are isolated, purified to homogeneity and biochemically characterized [17–19,41].

In contrast to all staphylococcal lipases previously described, a newly isolated SXL2, SXL3 and SAL3 exhibit a high stability at elevated temperatures.

In addition to its stability at high temperature, the SAL3 is an alkaline enzyme and remains stable between pH 5 and pH 12 after 24 h of incubation time. Besides, this lipase exhibited temperature and pH suitable for detergent formulation, the compatibility of SAL3 in the presence of some surfactants was checked. SAL3 is highly stable towards some known surfactants and retained its full activity in the presence of Triton TX-100, NaDC, NaTDC and Tween 80 (Table 2). The compatibility of SAL3 in the presence of some commercial detergents was also studied. This enzyme retained 100% of its activity in the presence of Axion, Ariel or Omino Bianco. To allow further comparison, the stability of other staphylococcal lipases (SSL and SXL1) was checked. Results show that, like SAL3, SSL and SXL1 are also stable in the presence of some surfactants, detergents and oxidizing agents.

Considering the overall properties of different staphylococcal lipases, one can say that these lipases can be considered as a potential candidate to be used in biotechnology and essentially for application in the detergent industry.

Table 2
Lipase stability of SAL3, SXL1 or SSL in presence of surfactants, detergents and oxidizing agents (1%w/v or v/v).

Surfactants/detergents/ oxidizing agents	Residual activity (%)				
	SAL3	SXL1	SSL	BCL	Lipolase
Control	100	100	100	100	100
Surfactants					
Triton X-100	100	83	33	93	67
Tween-80	100	95	58	40	49
Sodium cholate	100	41	50	93	110
Sodium taurocholate	100	37	33	80	92
Detergents					
Ariel	100	38	25	67	40
Axion	100	61	8	–	–
Omino bianco	100	38	41	–	–
Oxidizing agents					
Hydrogen peroxyde	60	34	0	100	80
Sodium perborate	70	53	0	100	100

Data are derived from Horchani et al. [18] and Saxena and Gupta [102].
BCL, *Burkholderia cepacia* lipase.

Table 3
Immobilization yield of staphylococcal lipases into $CaCO_3$.

Lipases	Immobilization yield (%)	Load (IU/g)	References
SSL	52	1300	[42]
SXL1	47	1450	[22]
SAL3	79	1500	[20]
SXL2	82	3250	[46]
ROL	95	2570	[42]
PSL	57.8	–	[44]
CRL	3.5	–	[45]

PSL: *Pseudomonas* sp. KWI65L lipase.
CRL, *Candida rugosa* lipase.

3.2. Increase of the stability of staphylococcal lipase after immobilization

One can note that the main hurdle to the use of free lipase in detergent or in ester production is the cost of biocatalysts. In addition, the conversion yield of the ester synthesis using free lipases was rather low due to the lipase inhibition by organic solvents or short chain acid used in the reaction medium. This inhibition can be successfully tackled by the immobilization of the enzyme. In fact, the use of immobilized lipases in industrial applications is very important since they can be recycled, which reduces catalyst costs.

Immobilization of lipases was previously studied [42,43]. It has been achieved by adsorption onto support matrices such as particles, fibres, by entrapping them in gel matrices and by covalent attachment. The $CaCO_3$ was selected as the most suitable adsorbent used to immobilize staphylococcal lipases since it preserve the catalytic activity almost intact and offer maximum adsorption capacity. A high immobilization yield (47–82%) was obtained after immobilization of staphylococcal lipases by adsorption into the carbonate of calcium (Table 3). The same support was found to be suitable for immobilization of many other microbial lipases, like the *Rhizopus oryzae* lipase [42] and the *Pseudomonas* SP KWI 56 lipase [44], with an immobilization yield of about 93.7% and 57.8%, respectively [42,44].

The biochemical properties of immobilized lipases were compared to those of the free ones. The results show that, after immobilization, the activity as well as the stability of the staphylococcal lipases was highly improved. Furthermore, we have shown that after immobilization, the SAL3 displayed a residual activity of 88, 81 or 78% after 60 min of incubation at 70, 80 or 90 °C, respectively, in contrast to free lipase which is fully inactive beyond 70 °C. At 100 °C, the half life of immobilized SAL3 was 55 min. Similar results were obtained when immobilizing the SXL1 and the SXL2 into $CaCO_3$ showing that the immobilized lipases were more stable than the free ones [42,43,46,47]. The increase of the thermal stability of the immobilized lipases compared to the free ones was also shown when immobilizing the *R. oryzae* lipase into $CaCO_3$ [43]. In fact, after 24 h of incubation at 50 °C, the free lipase was completely inactivated while the immobilized lipase displayed a residual activity of about 67%. At 60 °C, the immobilized lipase retained 32% of its initial activity while the free one was fully inactivated [43].

Recently a new lipase from *Staphylococcus warneri* EX17 was isolated and its immobilization was tested using three different protocols: adsorption on hydrophobic supports, mild and multipoint covalent attachments. It has been shown that the low stability of the enzyme at pH 10, which is a condition required to immobilize the enzyme on glyoxyl-agarose, could be improved by the addition of some polyols such as 25% of glycerol. After the immobilization into glyoxyl-agarose at pH 10, the enzyme retained about 80% of its initial activity [48]. Immobilizations on cyanogen bromide have no effect on the enzyme activity, while immobilization on octyl-sepharose increased the enzyme activity 1.6 times.

The high activity and stability of immobilized staphylococcal lipases are a potential advantage for practical applications of these enzymes.

3.3. Sn-2 preference of staphylococcal lipases

Intensive research is being carried out to make use of the specificities and preferences of some lipases for synthesizing lipids with highly specific fatty acid compositions and patterns of distribution, which could be used in the production of human dietary supplements and edible oils [49,50]. Few lipases show any preference for acylglycerol in the sn-2 (or internal) position. A minor component of *Candida antarctica* lipase [51,52] and *Geotrichum candidum* lipase [34] were reported to show sn-2 preference during hydrolysis. A review on CALA illustrates the increasing attention being paid to this unique biocatalyst and the many applications which have been developed, especially in the synthesis of structured triacylglycerol [53]. It has been found in several studies that CALA is the only lipase able to catalyze esterification reactions of highly sterically hindered substrates. Recently, we have shown that many staphylococcal lipases efficiently hydrolyzed the secondary ester group of diglyceride analogs as well as triolein at the sn-2 position [54]. These results indicate that staphylococcal lipases may open up for the use of lipase catalysis in reactions that are normally not considered compatible with the use of enzymes due to steric hindrance. This interesting property is highly sought by industrials to use lipases having a preference to sn-2 position to synthesize many specific compounds.

3.4. Production of ester by staphylococcal lipases

3.4.1. Stapylococcal lipase used as starter culture in fermented sausage

Strains of *Staphylococcus* added as starter culture in fermented sausage participate in the development of typical flavor [55,56]. They influence the level of many fragrant compounds such as esters, which have low sensory threshold values and will impart fruity notes to the sausage flavor [57,58]. Most of the esters in sausages are ethyl esters, their production depending on the presence of ethanol and different acids as well as on the esterase/lipase activities of the strains.

Staphylococcus specie is one of the main bacterial found in most naturally fermented sausages. The ability of resting cells and extracellular concentrates containing lipases to synthesize esters was studied in the presence of ethanol and organic acid with different chain length. The results show that *Staphylococcus* strains had a high potential for the esterification either by their resting cells or their extracellular concentrates [59]. Their lipolytic activities are very interesting during sausage manufacturing.

In addition to the use of the free staphylococcal lipases as starter in fermented sausage, immobilized lipases are used as biocatalysts to synthesize other esters such as monoglycerides, isoamyl aceteate, valerate and hexyl acetate, which can be used in many fields.

3.4.2. Monoglyceride synthesis

Monoglycerides are nonionic surfactants and emulsifying molecules with both hydrophilic and hydrophobic regions. They are widely used in food emulsifiers for bakery products, margarines, dairy products and sauces [60]. In the cosmetic industry, they are added as texturing agents for improving the consistency of creams and lotions [61,62]. In addition, owing to their excellent lubricant and plasticizing properties, monoglycerides are used in textile processing, formulation of oil for different types of machinery [63]. Currently, monoglycerides are manufactured on an industrial scale by continuous chemical glycerolysis of fats and oils at high temperature (220–250 °C) employing alkaline catalysts under a nitrogen gas atmosphere [64]. The products obtained by this procedure have several drawbacks (dark color and burnt taste) and the yield is rather low. To overcame the disadvantage of chemical process, a direct esterification of oleic acid with glycerol in a solvent free system using immobilized *Staphylococcus simulans* lipase as catalyst was realized by Ghamgui et al. [65] (Table 4). The optimal conditions for the monoolein synthesis were determined. A conversion yield of 70% was obtained using an amount of lipase of 100 IU, an initial added water of 5% (w/w) and an oleic acid to glycerol molar ratio of 0.2. Many researchers have reported the use of microbial lipases in the synthesis of monoolein by direct esterification of oleic acid with glycerol. For example, the *Penicillium camembertii* lipase immobilized on epoxy SiO2-PVA was used in the monoolein synthesis in solvent-free media with a yield of conversion about 39.9% [66]. Yamaguchi and Mase have obtained a conversion yield of synthesis of monoolein of about 76% using the mono- and diacylglycerol lipase (MDGL) from *Penicillium camembertii* U-150 [67].

3.4.3. Isoamyl acetate synthesis

The immobilized *S. simulans* lipase was also used to catalyze the esterification reaction between acetic acid and isoamyl alcohol to synthetize isoamyl acetate (banana flavour) in pure substrate conditions (without addition of any organic solvent). The optimized conditions for the synthesis of isoamyl acetate were determined to 60 IU of immobilized lipase, an acid/alcohol of 2 molar ratio and initial added water of 10% (w/w) at 37 °C and 200 rpm. In such conditions, the reached conversion yield was about 64% during 8 h of incubation. The isoamyl acetate production have also been investigated in a solvent free system by Güvenc et al. who have achieved an isoamyl acetate conversion of 80% using 5% (g enzyme/g substrates, w/w) of *C. antarctica* lipase (Novozyme 435) with 3.6 M acid concentration (acid/alcohol molar ratio of 1/2) at 30 °C in 6 h [68].

3.4.4. Ethyl valerate and hexyl acetate synthesis

The production of ethyl valerate and hexyl acetate, two aromatic molecules, was also carried out using immobilized *S. simulans* lipase in a solvent free system. These two aromatic molecules are among the important and versatile components of natural flavors and fragrances [69]. Ethyl valerate with a typical fragrance compound of green apple and hexyl acetate with a pear flavour property are in high demand and are widely used in food, cosmetic and pharmaceutical industries. Different reaction parameters for enhancing ester formation were investigated. In fact, higher conversion of ethyl valerate (51%) was achieved by the addition of 20% (w/w) of water to the reaction mixture, using 200 IU of immobilized lipase, a molar ratio valeric acid to ethanol of 1 and at 37 °C. No decrease of the synthesis activity was observed after 10 cycles of use of the immobilized lipase. Bayramoglu et al. (2011) have also investigated ethyl valerate synthesis by immobilized *Candida rugosa* lipase in solvent free system and in hexane [70]. It was found that the maximum esterification yield obtained was 34.8% or 67.2% in solvent free system or in hexane, respectively. From these results, one can note that the immobilized *S. simulans* lipase is more efficient than the *C. rugosa* lipase in the synthesis of ethyl valerate in solvent free system. In the case of the hexyl acetate, the optimum conditions, giving 41% of conversion, are obtained with 10% (w/w) of initially added water, 100 IU of immobilized lipase, a molar ratio acetic acid to hexanol of 1 and at 37 °C. Immobilized lipase could be repeatedly used for five cycles without a decrease of synthesis activity. Shieh and Chang have achieved an hexyl acetate conversion of 86.6% by transesterification of hexanol with triacetin in n-hexane using the immobilized *Mucor miehei* lipase (Lipozyme IM-77). The optimum synthesis conditions used were as follows: reaction time, 7.7 h;

Table 4
Some esters synthesized by staphylococcal lipases.

Esters	Biological activity	Lipases	Conversion (%)	Other notes	References
Monoolein	Emulsifying molecule	SSL	70	– The highest conversion yield of	[65]
		Penicillium camembertii lipase	39.92	monoolein synthesis in solvent-free system was obtained	[66]
		Penicillium camembertii U-150 lipase (MDGL)	76	with SSL	[67]
Isoamyl acetate	Banana flavour	SSL	64		[65]
		Candida antarctica lipase (Novozyme 435)	80		[68]
Ethyl valerate	Green apple flavor	SSL	51	– The highest conversion yield of	[47]
		Candida rugosa lipase	34.8 (Solvent free system) 67.2 (hexane)	ethyl valerate synthesis in solvent-free system was obtained	[70]
Hexyl acetate	Pear flavor	SSL	41	with SSL	[47]
		Mucor miehei lipase (Lipozyme IM-77)	86.6		[71]
Decyl esters	Flavoring	SEL (decyl laurate)	59.5	– The SEL was the first microbial lipase used for the synthesis of decyl esters in solvent-free system	[75]
		Candida antarctica Lipase B (Novozyme 435) (Decyl acetate)	100	– The highest conversion yield of decyl laurate synthesis in solvent-free system was obtained with SEL	[76]
Butyl acetate	Pineapple flavor	r-SXL1	80	– The immobilized SXL1 and its	[72]
		LK-SXL1	95	mutant (LK-SXL1) are the most	
		Rhizopus oryzae lipase	76	powerful enzymes for the	[73]
		Rhizopus oligosporus lipase	54.6	synthesis of butyl acetate.	[74]
Tyrosol acetate	Antioxidant	SXL1	95.3	– The highest conversion yield of tyrosol acetate synthesis in solvent-free systemwasobtained	[22]
Propyl gallate	Antioxidant	*Candida antarctica* lipase	95	with SSL	[81]
		SXL2	90	– The SXL2 was the first microbial lipase used for the synthesis of propyl gallate	[46]
		Aspergillus niger Van Teighem	86	– The highest conversion yield of propyl gallate synthesis in solvent-free system was obtained with SXL2	[83]
Butyl oleate	Biodiesel fuel	SAL3	71		[92]
		Rhizopus oryzae lipase	75 (solvent free system) 73 (Hexane)		[42]
Starch esters	Biopolymer	SAL3 (Starch oleate)	75	– The highest conversion yield of	[20]
		Thermomyces lanuginosa lipase (Starch laurate)	52.88	starch oleate synthesis was obtained with SAL3	[100]
		Candida rugosa lipase (Starch laurate)	55.28		[101]
Eugenol Benzoate	Antioxidant	SAL3	76	– The enzymatic synthesis of eugenol benzoate is carried out for the first time with SAL3	[21]

temperature, 52.6 °C; enzyme amount, 37.1%; substrate molar ratio, 2.7:1; and added water, 12.5% [71].

3.4.5. Butyl oleate synthesis

The high conversion yield was obtained when using immobilized *Staphylococcus xylosus* lipase (80%) and its mutant (LK-SXL1) (95%) as biocatalysts to catalyze the esterification reaction between acetic acid and butanol to produce butyl acetate ester (pineapple flavor) (Table 4), which can be used in food, cosmetic and pharmaceutical industries [72]. Butyl acetate was previously synthesized with *R. oryzae* lipase immobilized onto $CaCO_3$ [73]. The maximum conversion yield reached was 76% after 24 h of incubation time using a higher enzyme amount (500 UI). Furthermore, Mahapatraa et al. have optimized the enzymatic synthesis of butyl acetate using *Rhizopus oligosporus* lipase (NRRL 5905) immobilized onto cross-linked silica gel 60. A conversion yield of 54.6, using 27.5% enzyme concentration for 28 h of incubation was achieved [74]. Following this comparison, one can note that the immobilized *S. xylosus* lipase and its mutant (LK-SXL1) are the most powerful among these enzymes cited for the synthesis of butyl acetate.

3.4.6. Decyl ester synthesis

Various flavor esters were also obtained by using recombinant lipases from *Staphylococcus epidermidis* as a catalyst in an aqueous environment [75]. These esters were enzymatically synthesized to overcome the problems associated with chemical processes. Results show that the *S. epidermidis* lipases could catalyze ester synthesis (59.5) from decyl alcohol and fatty acids of different chain length (Table 4). Decyl esters were also synthesized by other microbial lipases like the *C. antarctica* Lipase B (Novozyme 435) immobilized in a macroporous resin which catalyzes the synthesis of decyl acetate ester in supercritical carbon dioxide by transesterification of vinyl acetate with decanol [76]. Although the difference of the conversion yield obtained when using the two lipases, one can note that the recombinant lipases of *S. epidermidis* are particularly interesting because they allow catalysis of ester synthesis without organic solvents. This system presents the following advantages: (i) avoiding the problem of toxicity and flammability of organic solvents; and (ii) simplification of product purification conditions.

The use of immobilized staphylococcal lipases appears to be quite attractive in a variety of flavor ester preparations under milder conditions and the product may obtain the “natural” label.

3.5. Production of antioxidants by staphylococcal lipases

Polyphenolic compounds produced by plants are of considerable research interest, both as functional food ingredients and as nutraceuticals, because of their antioxidant properties [77] and other beneficial biological activities.

Different antioxidant molecules were successfully synthesized using staphylococcal lipases as catalysts (Table 4).

3.5.1. Tyrosol acetate synthesis

A recent increase in serious research on the commercial application of radical scavengers as beneficial anti-ageing and photoprotection ingredients in cosmetic products [78], and the demand for non-toxic antioxidants that are active in hydrophilic and lipophilic systems, led to the additional focus on a new natural antioxidants that can be used in oil-based formulas and emulsions. The acetylation of biophenols increase their hydrophobicity and therefore, lipid solubility and may modify their bioavailability [79], antioxidant effect in food emulsions [80], stability and color stability.

The immobilized SXL1 was used to catalyze the transesterification of tyrosol and ethyl acetate [22]. Response surface methodology (RSM) was used to evaluate the effects of the temperature, the enzyme amount and the ethyl acetate/hexane volume ratio on the tyrosol acetylation conversion yield. A statistical model predicted that the highest production yield of acetylated tyrosol was 96.86% ± 3.7 for the following reaction conditions: a reaction temperature of 54 °C, an enzyme amount of 500 UI and a volume ratio ethyl acetate/hexane of 0.2. Two independent replicates were carried out under the optimal conditions predicted by the model. The maximum conversion yield reached 95.36% ± 3.6 which agreed with the expected value. The ester obtained was characterized by spectroscopic methods. Chemical acetylation of tyrosol was performed and the products were separated using HPLC. Among the eluted products from HPLC, mono and diacetylated derivatives were identified by positive mass spectrometry [22]. Tyrosol and its monoacetylated derivative exert similar antiradical activity on 2,2-diphenyl-1-picrylhydrazyle. Similar results were obtained by Grasso et al. who have achieved an acetylated tyrosol conversion of 95% by transesterification of vinyl acetate with hydroxytyrosol in t-butylmethyl ether using *C. antarctica* lipase [81].

3.5.2. Propyl gallate synthesis

Propyl gallate is used as a synthetic antioxidant in processed food, cosmetics and food packing materials, to prevent rancidity and spoilage. It is also used to preserve and stabilize medicinal preparations on the US Food and Drug Administration list [82].

The immobilized SXL2 was also used to catalyze the esterification of propanol with gallic acid in solvent-free system [46]. The optimum conditions of the reaction were determined by RSM. A high conversion yield was obtained and reached 90%. There are no further studies that report the use of microbial lipases in propyl gallate synthesis. However, this reaction was carried out by other kinds of enzymes as biocatalysts. For example, Sharma and Gupta have achieved a propyl gallate production of 86% using the tannase from *Aspergillus niger* [83]. Furthermore, Xiao and Yong have studied the kinetics of propyl gallate production using the *A. niger* mycelium-bound tannase [84].

3.5.3. Eugenol benzoate synthesis

Several previous studies have shown that eugenol has the beneficial antioxidant and anti-inflammatory activities, which stem from the inhibition of prostaglandin synthesis, neutrophil chemotaxis, and pyretic activity [85–88]. In addition, eugenol has an antigenotoxic and anticarcinogenic potential [89,90]. When the hydroxyl group of eugenol is protected by a bulky and moderately lipophilic group (like benzoate), the obtained ester can be acted as lipoxygenase inhibitor. Recently several eugenol esters were chemically synthesized and evaluated as potential inhibitors of lipoxygenase [91]. The benefits of these compounds could be enhanced with the improvement of an efficient method for their synthesis under mild conditions. There are no further studies that describe the synthesis of eugenol esters using lipases. To overcome the disadvantages of the chemical processes, we have used for the first time a staphylococcal lipase (SAL3) as catalyst to synthesize the eugenol benzoate (Table 4). A high conversion yield was obtained and reached 75% using the immobilized SAL3 as biocatalyst [21]. This may have an important impact on the cost of the ester production and encourage use of natural compounds such as eugenol. The optimized conditions for the synthesis of the eugenol benzoate using RSM were reported. Furthermore, we have shown that the synthesized ester presents a high antioxidant activity slightly less pronounced than that of eugenol. The synthesis of eugenol derivates via enzymatic esterification of hydroxyl function with a bulky and moderately lipophilic group, such as benzoic acid, can be used as a tool to increase the lipophilic character of eugenol. Moreover, these lipophilic analogs could be used as food antioxidant, flavors, perfumes and pharmaceuticals fields.

3.6. Production of biofuel by staphylococcal lipases

Lipases have become one of the most important groups of enzymes for its applications in organic synthesis. They can be used as biocatalyst in the production of useful biodegradable compounds. The main hurdle to the use of lipase for biodiesel fuel production is the cost of biocatalysts. As a means of reducing the cost, the use of immobilized lipase within support is significantly advantageous. Immobilized SAL3 was used to catalyze the esterification of 1-butanol with oleic acid [92]. The reaction product, 1-butyl oleate, is useful as a diesel additive, a polyvinyl chloride plastisizer, a water-resisting agent. A high reaction yield (71%) was obtained using 100 IU and a concentration of oleic acid and butanol of 0.1 M. Similar conversion yields were obtained for the synthesis of 1-butyl oleate in solvent free system and in n-hexane by the *R. oryzae* lipase immobilized on $CaCO_3$ [42]. Linko et al. have also reported the use of 25 commercial lipases in butyl oleate synthesis and have found that butyl oleate was produced at high yields from oleic acid and 1-butanol by lipases from *C. rugosa*, *Chromobacterium viscosum*, *Rhizomucor miehei* and *Pseudomonas fluorescens* [93].

3.7. Production of biopolymers by staphylococcal lipases

The introduction of an ester group into polysaccharide constitutes an important achievement because it resulted in modifying their original hydrophilic nature and obtaining new thermal and mechanical properties [94]. Previous works have reported the use of organic solvents to achieve starch solubilization followed by its esterification, to produce fully biodegradable thermoplastic materials [95–97]. Such compounds can replace the non-biodegradable plastics used in the plastics industry. This achievement would help to save petrochemical resources and to find out new industrial uses of starches. The biodegradable product obtained after esterification has biomedical applications such as carriers for controlled drugs release and other bioactive agents [98]. However, the prepared esters were synthesized by chemical gelatinization using formic acid, followed by treatment with fatty acid chlorides. Such methods rely on the use of sophisticated experimental techniques. In addition, the utilization of organic solvent is prohibited for industrial applications, especially in food industries. In fact, using lipase as biocatalyst eliminates the disadvantages of the chemical process by producing very high purity compounds with less or no downstream operations.

It has been previously reported that microwave heating can be used to synthesize biopolymers and accelerates the esterification of starch without any activation of further specific bonds [99]. However, the use of this technique needs a highly stable lipase. The immobilized SAL3 was chosen to catalyze the esterfication of starch with oleic acid [20]. The stability of immobilzed and free SAL3 under microwave heating was determined. Results show that the immobilized SAL3 exhibited more than 70% of its initial activity during 30 min of incubation time under microwave radiation. This lipase was used as catalyst to synthesize the starch oleate since the esterification conditions were optimized by RSM. The optimized conditions were 386 IU of immobilized lipase, an oleic acid/starch molar ratio of 0.18 at 44 °C and 200 rpm. Under these conditions, 76% of conversion with a degree of substitution of 2.86 was reached after 4 h of incubation [20]. Lipases from *Thermomyces lanuginosa and C. rugosa* were used as biocatalysts in the starch esterification with hydrolyzed coconut oil [99,100]. In microwave oven esterification, maximum degree of substitution of 1.55 and 1.1 were obtained, respectively. From these examples, one can note that SAL3 presents a better effectiveness in starch esterification than those of *T. lanuginosa* and *C. rugosa* lipases.

4. Conclusion

The use of staphylococcal lipases for a variety of biotechnological applications is rapidly and steadily increasing. Several novel staphylococcal lipase genes are still to be identified and enzymes with new and exciting properties will be discovered. In parallel, the combination of optimized lipases with improved reaction conditions will lead to novel synthetic routes, allowing the production of high-value chemicals and pharmaceuticals.

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