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Burkholderia cepacia lipase: A versatile catalyst in synthesis reactions†

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Abstract

The lipase from *Burkholderia cepacia*, formerly known as *Pseudomonas cepacia* lipase, is a commercial enzyme in both soluble and immobilized forms widely recognized for its thermal resistance and tolerance to a large number of solvents and short-chain alcohols. The main applications of this lipase are in transesterification reactions and in the synthesis of drugs (because of the properties mentioned above). This review intends to show the features of this enzyme and some of the most relevant aspects of its use in different synthesis reactions. Also, different immobilization techniques together with the effect of various compounds on lipase activity are presented. This lipase shows important advantages over other lipases, especially in reaction media including solvents or reactions involving short-chain alcohols.

Key words: lipase; synthesis reactions; lipase immobilization; Amano Lipase PS

1. Enzymes in biotechnological processes.

Enzymatic catalysis is widely used in the synthesis of numerous products (Liese et al., 2006). The synthesis and enzymatic modification of compounds have two main advantages: first, obtaining the desired products in high proportions due to the specificity and selectivity of some enzymes, and second, the low purification requirements due to little or no generation of by-products. Other advantages of enzymes (in addition to their specificity) are the ability to operate under mild reaction conditions, availability from a wide range of sources, and the improvement of the enzyme activity by changes in the reaction medium or using different immobilization techniques (Gupta et al., 2003).

Lipases are naturally designed to act at an oil-water interface, which makes them very compatible with organic solvents (Jaeger and Eggert, 2002; Madeira Lau et al., 2000; Hari Krishna and Karanth, 2002; Reetz, 2002; Gotor-Fernández et al., 2006; Hasan et al., 2006). These enzymes may act in different reaction media, recognize a wide variety of substrates and catalyze a large number of reactions, such as hydrolysis (Charusheela and Arvind, 2002; Vaysse et al., 2002; Liu et al., 2016; Fernandez-Lorente et al., 2001), esterifications (Vaysse et al., 2002; Zaidi et al., 2002; Gandhi et al., 2000; Kontogianni et al., 2003), alcoholysis (Vaysse et al., 2002; Soumanou and Bornscheuer, 2003; Shimada et al., 2002; Deng et al., 2005), ammoniolysis (López-Serrano et al., 2001; Levinson et al., 2005; De Zoete et al., 1996; Gotor-Fernandez and Gotor, 2006), aminolysis (Torre et al., 2005; Badjic et al., 2001; Gotor-Fernandez and Gotor, 2006), transesterification (Katiyar and Ali, 2012; Katiyar and Ali, 2015), interesterification (Xu and Liu, 2005; Yang et al., 2003; Zhang el al., 2000; Abigor et al., 2003) and others.

Lipases are the most recognized group of biocatalysts in biotechnology (Jaeger and Eggert, 2002; Hasan et al., 2006; Houde et al., 2004). Their versatility make these enzymes very attractive for applications in a variety of industries such as food, pharmaceutical, detergent, leather, textile, cosmetic, and paper (Kirk et al., 2002; Sharma et al., 2001). Lipases are

carboxyl-esterases that act on acylglycerides. Their catalytic triad is composed of serine, histidine and aspartate or glutamate, which is also found in serine proteases (Wallace et al., 1996; Wallace et al., 1997; Cai et al., 2004).

Lipases have different degrees of selectivity to the substrates, and the reaction rate is directly related to the structure thereof (Jensen et al., 1983). Lipases are generally classified according to their regiospecificity toward positions of the glycerol backbone. They can be 1,3-specific or 1,2,3-specific (Jensen et al., 1983; Berger et al., 1992a; Sugihara et al., 1994). Lipases can also show selectivity toward different types of fatty acids, in regard to carbon chain length or degree of unsaturation (Jensen et al., 1983; Kirk et al., 1992).

The main structural feature of lipases is the existence of an amino acid chain that covers the active site, called lid or flap. The lid is a variable-length string for each lipase, and its movement gives access to the substrates to the active site in a phenomenon called "interfacial activation" (Verger, 1997; Martinelle et al., 1995; Brzozowski et al., 2000; Louwrier et al., 1996; Secundo et al., 2006). The movements of the lid are given in response to the characteristics of the reaction medium. In aqueous media, lipases have their active center secluded from the medium by the lid. In a nonpolar medium, the lid moves allowing the entry of the substrates to the active site of the enzyme (Belle et al., 2007; Cajal et al., 2000; Peters et al, 1996; Ericsson et al., 2008).

In this review, we will focus on the uses given to the lipase from *Burkholderia cepacia* (BCL) (formerly known as Pseudomonas cepacia lipase) as catalyst in the synthesis and modification of various products, as well as on studies on the selectivity of the enzyme and simple techniques for improving the activity and stability of the biocatalyst.

1.1. Some properties of *Burkholderia cepacia* lipase

BCL is an extracellular enzyme, and it is one of the most widely used biocatalysts in biotechnological processes. The extensive use of this lipase is due to its ability to recognize a

wide variety of substrates, heat resistance, and tolerance to multiple solvents, including short-chain alcohols (Bornscheuer et al., 1994a; Sasso et al., 2016).

BCL exhibits high hydrolytic activity towards triglycerides regardless of the chain length of the fatty acids. This enzyme does not have a particular positional specificity (Kim et al., 1992). It is available from Amano Enzyme Inc. in free form (Amano Lipase PS SD) and two immobilized forms: Amano Lipase PS-D (immobilized on diatomite) and Amano Lipase PS-C (immobilized on ceramic particles). BCL in free form is a yellowish or white powder, soluble in water, and it is diluted with dextrin. This product is widely used for separation of optically active compounds.

BCL was cloned and its amino acid sequence was elucidated from complementary DNA sequence (cDNA) (Jørgensen et al., 1991). It was also characterized (Ihara et al, 1991; Sugihara et al, 1992; Bornscheueret al, 1994) and crystallized (Kim et al, 1992; Bornscheuer et al, 1994; Kim et al, 1997). Its polypeptide chain consists of 320 amino acid residues with a calculated molecular mass of 33128 Da.

The active site includes Ser87, His286, and Asp264 (Fig. 1). Serine of the catalytic triad is located at the bottom of a cleft in the protein and is exposed to the solvent. Mainly hydrophobic residues constitute the walls around the active site (Frenken et al., 1992; Lang et al., 1996; Barbe et al., 2009; Schrag et al., 1997; Noble et al., 1993). Figure 1 shows the structure of BCL in its open-active conformation. The catalytic triad in the active site is represented by red sticks. The conformational changes of BCL during the phenomenon of interfacial activation are associated with the displacement of α5 helix (the lid) accompanied by the reorientation of α9 helix.

Insert Figure 1

The optimum temperature for this lipase was reported at 50 °C, but BCL maintained its activity even at 75 °C (Sugihara et al., 1992).

The stereo- and regioselectivity of BCL have been widely examined (Schulz et al., 2000; Tafi et al., 2000; Lang et al., 1998; Weissfloch and Kazlauskas, 1995; Ferraboschi et al., 1994). On the other hand, various authors have tried to relate conformational changes of BCL to its selectivity. Chemical modifications were performed in order to affect the selectivity of the lipase (Nguyen et al., 1997; Tuomi and Kazlauskas, 1999; Bianchi et al., 1993). The enantiopreference of BCL was modified using mutation techniques (Koga et al., 2003). These techniques were also used in order to affect its preference for any type of fatty acid (Yang et al., 2002).

Immobilization of lipase and bio-imprinting with substrate analogs are techniques designed to improve the activity and stability of the enzyme. Improved thermal stability and increased resistance to solvents and alcohols have been reported by several authors (Pencreac'h and Baratti, 1997; Liu et al., 2011; Cao et al., 2009).

2. Immobilization and improvement of the catalytic properties

2.1. Immobilization methodologies

BCL in its free form usually has low stability and its recovery is complex, making it virtually impossible to reuse. These features reduce the potential use of this lipase for practical purposes (Salum et al., 2008). Thus, the immobilization of lipase can not only improve its stability (and in some cases its activity), but also enable its recovery and reuse.

Methods of BCL immobilization include adsorption, covalent attachment, entrapment, cross-linked enzyme aggregates (CLEAs) and cross-linked enzyme crystals (CLECs) (Hara et al., 2008; Jegannathan et al., 2009; Cao et al., 2009). In addition to immobilization, molecular bioimprinting and interfacial activation are techniques for increasing the activity and stability of lipases (Foresti et al., 2005).

2.1.1. Immobilization by physical adsorption

Physical adsorption is a simple and widely used technique for carrying out the immobilization of enzymes .

Pencreac'h and Baratti (1997) immobilized BCL on microporous polypropylene powder Accurel EP-100 with a simple methodology. The authors reported an increase in lipase activity after immobilization. The relationship between the activity of the free and immobilized enzyme is an extremely important parameter for assessing the immobilization process. The biocatalyst was used for the hydrolysis of p-nitrophenyl palmitate (pNPP) and p-nitrophenyl acetate (pNPA). In both reactions the authors reported an unusual high increase in lipase activity.

Immobilization by adsorption onto a polymer matrix was also studied by Dhake et al. (2013). In this case, the support was a copolymer based on polyurethane and β-cyclodextrin (β-CD). The copolymers contained β-CD with two types of crosslinker units: 4,4´-dicyclohexylmethane diisocyanate (CDI) and 4,4´-diphenylmethane diisocyanate (MDI), respectively. The biocatalysts obtained were tested in the hydrolysis of pNPP, showing greater activity than free lipase (three times higher for CDI and four times higher for MDI). The high hydrolytic activity of the biocatalyst remained constant in a pH range of 5 to 9, temperatures of 25 to 65 °C and in different nonpolar solvents.

Immobilization of BCL on mesoporous silicates with functionalized surfaces was conducted by Kato and Seelan (2010). The surface functionalization with phenyl groups increased the adsorption capacity of the support. Biocatalysts were evaluated in hydrolysis and acetylation reactions, and the performance was compared with those obtained for the free lipase. The biocatalysts showed high activity in both reactions. Another study using functionalized mesoporous silicates was presented by Jin et al. (2011). In this work, the functionalization was done with hydrophobic groups. Biocatalysts were evaluated in transesterification reactions and hydrolysis. The activity of the biocatalysts was considerably higher than that observed for

the free lipase, probably because the hydrophobic groups of the support surface promoted the "interfacial activation" of the lipase.

Liu et al. (2011a) not only studied the immobilization of BCL on a crosslinked polystyrene (NKA resin), but also improved the catalytic activity by combining strategies of bioimprinting and interfacial activation. BCL was incubated for 60 min in a mixture of isopropanol and bioimprinting molecules (decanoic acid, lauric acid, myristic acid, palmitic acid, and stearic acid), and after the set time, the bioimprinting molecules were removed with octane. After incubation, the bioimprinted lipase was immobilized by physical absorption on NKA resin. Finally, immobilized–bioimprinted BCL was incubated for 60 min at room temperature in nonpolar organic solvents (n-hexane, cyclohexane, isooctane and n-heptane) to promote interfacial activation. The biocatalyst obtained showed good thermal stability, high resistance to solvents (particularly ethanol, methanol, and acetone) and it maintained 92% of its initial activity after being used in 50 successive reaction batches of 8 hours each.

Immobilization by physical adsorption is a simple and widely used technique that allows the binding of the enzyme to a support through van der Waals, dipolar and H-bonding bonds. These bonds do not affect the structure of the protein and therefore their activity is not affected, however, the desorption of lipase (or leaching) is the main problem in these biocatalysts.

2.1.2. Immobilization by entrapment

Entrapment involves the capture of the enzyme within a matrix (Roy et al., 2004). The main features that have turned this method into one of the most widely applied techniques are the simple and repeatable process, mild reaction conditions and low denaturation of the enzyme (Mohapatra and Hsu, 2000).

Jagannathan et al. (2009) reported the immobilization of BCL by entrapment within *κ*-carrageenan. *κ* -Carrageenan is a high molecular weight polysaccharide extracted from marine

red algae (Jang et al., 1996). This polymer was first converted into a gel and then joined lipase in powder form. The gel containing the lipase was hardened in KCl solution at 4 °C for 24 h. Finally, the solid was filtered, washed, dried and tested in the hydrolysis of pNPP. The biocatalyst was active and stable at 50 °C and pH between 6 and 9. After 6 reaction batches, the entrapped lipase maintained 72.3% of its initial activity.

2.1.2.1. Entrapment of cross-linked aggregates of lipase

Cross-linked enzyme aggregates (CLEAs) are not immobilized enzymes, they are the result of protein precipitation followed by crosslinking with a dialdehyde (Schoevaart et al., 2004; Cao et al., 2003). Entrapment of CLEAs has been proposed to improve the operating conditions of these enzyme aggregates. For example, the entrapment would avoid leaching of the enzyme to the reaction medium.

Liu et al. (2011b) studied the entrapment of CLEAs within a matrix sol-gel. First, BCL-CLEAs were obtained with the use of different precipitants and varying amounts of glutaraldehyde as the crosslinker. Then, CLEAs was entrapped within a sol-gel support. The biocatalyst generated with this method was tested in esterification and transesterification reactions. It exhibited high catalytic activity, obtaining values 1.7 and 13.2-times greater than those obtained with CLEAs without entrapment and free BCL, respectively. Similar results were reported by Abdullah and Ravindra (2013b). In this case, BCL-CLEAs were entrapped within a polymeric matrix composed of equal proportions of *κ*-carrageenan and alginate. As mentioned above, *κ*-carrageenan is a natural polysaccharide extracted from marine red algae, while alginate is an anionic polymer extracted from brown seaweeds. The effects of different parameters on the immobilization process were evaluated. The biocatalyst obtained was evaluated in the hydrolysis of olive oil. The authors reported that the entrapped BCL-CLEAs showed 89.26% of the activity recorded for the free lipase, however they were considerably more stable and maintained the activity after successive uses.

2.1.3. Immobilization by covalent bond

In the methods described above, possible leaching of the enzyme is a recurring problem. The application of biocatalysts obtained by physical adsorption or entrapment is also limited by the reaction medium (Moreno et al., 1997; Mustranta et al., 1993). The covalent-bonded immobilization would allow a stronger bond, preventing loss of the enzyme and achieving greater long-term stability (Moreno et al., 1997). This method involves the reaction of chemical groups of the support with nucleophiles of the proteins (lysine, cysteine, tyrosine, histidine, methionine, etc.) (Arroyo, 1998).

Yemul and Imae (2005) studied the covalent immobilization of BCL on dendrimers. These three-dimensional macromolecules have peripheral functional groups readily available for the immobilization of lipase. In this report, polyphenylene sulfide (PPS) dendrimer was used as support. The biocatalyst obtained was tested in the hydrolysis of olive oil, and it was active in a higher temperature and pH range (compared to the free lipase). The thermal stability of lipase was improved by the immobilization process, and the biocatalyst retained 90% of its initial activity after 20 successive batches.

Covalent immobilization of BCL on semiconducting materials was studied by Fernandez et al. (2008). Crystalline silicon, porous silicon, and these materials coated with silicon nitride were used as support lipase. The activity of the biocatalysts was evaluated through hydrolysis of pNPP. The results indicated that more than twice the amount of lipase was immobilized on porous silicon than on crystalline silicon. These results are probably due to the increased surface area of the porous silicon. In both cases the coating of the surfaces with silicon nitride increased the bonds with the proteins.

Li studied the covalent immobilization of BCL on electrospun polyacrylonitrile (PAN) nanofibrous membrane (Li et al., 2011a; Li et al., 2011b). First, the nitriles groups of PAN nanofibers were activated by an amidation reaction and then they were reacted with the

lipase solution. The biocatalyst thus generated was tested in transesterification reactions, and it had higher thermal stability and greater tolerance to wider pH ranges than free lipase. The immobilized lipase also retained 79% of the activity exhibited by the free lipase. Immobilized BCL was used in 10 successive batches without further changes in its specific activity.

2.2. Improvements in catalytic properties

The different immobilization techniques have allowed that biocatalysts present a greater thermal resistance and stability against changes in pH. However, these improvements generally occur at the expense of loss of enzyme activity. Different methodologies have been evaluated to increase the activity of the enzymatic catalysts. In this sense, the treatment with ionic liquids has shown to be effective for this purpose. Li et al., 2008 studied the behavior of several commercial biocatalysts in various systems with organic solvents and using 1-butyl-3-methylimidazolium hexafluorophosphate ($[C_4mim]PF_6$) as the co-solvent. Amano Lipase PS-C significantly increased its activity and regioselectivity using tetrahydrofuran (THF) with 5% (v/v) of ($[C_4mim]PF_6$) as solvent. The effect of different anions in ionic liquids on BCL activity was evaluated by Vidya and Chadha, 2009. The authors compared the changes in lipase activity in reaction systems containing 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4mim]BF_4$), ($[C_4mim]PF_6$), and 1-butyl- 3-methylimidazolium bis (trifluoromethylsulfonyl) imide ($[C_4mim]$ Tf_2N). The enzymatic activity was higher in the presence of hydrophobic ionic liquids ($[C_4mim]$ Tf_2N and $[C_4mim]PF_6$) than in hydrophilic ionic liquids ($[C_4mim]BF_4$). These results indicate that the nature of the anion could influence the catalytic activity of BCL. In a later publication, the authors presented a comparative study about the impact of ionic liquids and hexane on the enzymatic activity of BCL. The biocatalyst was more active in [Bmim]Tf2N than in hexane (in esterification and transesterification reactions) (Vidya and Chadha, 2010). The effect of ionic liquids and organic solvents on BCL activity was also reported by Pan et al., 2010. The results showed high activity in systems composed of ionic liquids and organic solvents in a ratio of 1: 1

(v/v). The highest values of enzyme activity were given by *tert*-butanol:[C_4mim][Tf_2N], *tert*-butanol:[C_6mim][PF_6], and benzene:[C_4mim][NO_3]. A study with similar results was presented by da Graça Nascimento et al., 2015 but using BCL immobilized in three different supports. Conformational studies via circular dichroism spectroscopy revealed changes in the α-helix content in these reaction systems. The increase of the enzymatic activity could be related to the decrease of the content of the α-helix that favors the open configuration (Pan et al., 2010). The colophilization of BCL with ionic liquids has also been evaluated as a methodology to increase the enzymatic activity. Lee and Kim, 2011 carried out the lyophilization of BCL together with various ionic liquids containing the PF_6 anion. In all cases, hyperactivation of the lipase was recorded. The authors reported activity values between 63 and 663 times higher than untreated lipase.

The colophilization of BCL has also been evaluated with other compounds, for example, cyclodextrins, monosaccharides, and disaccharides. Secundo and Carrea, 2005 reported increases in the activity of colyophilized lipases with sugars. Activity values up to 4.7 times higher than with sugar-free BCL were recorded using sugar/BCL ratios greater than 20:1 (w/w). Sugars could prevent conformational changes caused by lipase/lipase interactions. In this way, mono- and disaccharides could be used as additives to improve the performance of BCL in organic systems (Azizi et al., 2011).

The use of sub-/super-critical CO_2 has been evaluated as an alternative medium for reactions in non-aqueous systems. In these reaction media, it is possible to modify the activity/selectivity of the biocatalyst by varying the pressure and/or temperature. In addition, the recovery of the products is simple in these reaction systems. Celia et al., 2005 reported high stability of BCL in supercritical CO_2. The reaction rate increased with increasing pressure and the optimum was 10 MPa. A high residual activity was observed with increasing pressure. Residual activities with values of 89, 86, and 84% were obtained at 15, 20, and 25 MPa, respectively. Liu et al., 2013 reported an increase in the activity of 116% after incubation of

BCL for 30 min in supercritical CO_2 at 40 °C and 10 MPa. The residual activity was 105% after 30 min incubation in subcritical CO_2 at 35 °C and 6 MPa. The hyperactivation of BCL after incubation in sub-/super-critical CO_2 was also reported by Chen et al., 2013. The results of conformational analysis did not show changes in the primary structure of BCL. However, changes in the secondary and tertiary structure were detected. These changes were probably responsible for the increases in enzyme activity (Chen et al., 2013; Liu et al., 2013). Unfortunately, improvements in the activity occurred at the expense of reduced thermal stability and tolerance to organic solvents.

The pretreatment of BCL with different organic compounds has been evaluated to modify both the activity and the selectivity of the biocatalyst. Liu et al., 2010 studied the effect of several solvents with log P values between -0.24 and 2.9 and with different functional groups (acetone, isopropanol, n-hexane, acetonitrile, butanol, ethanol, toluene, and n-heptane). The enhancements in the esterification activity of BCL had the following relationship to the functional groups: C=O ≈ C≡N > C–C >> OH. A similar report was presented by Bi et al., 2015. In this work, the activity of BCL in the esterification of glycerol and oleic acid was evaluated as a function of the log P of the used solvents. In addition, the effect of the solvents on the positional selectivity of BCL was also reported. The highest enzymatic activity was obtained with the solvents with the highest Log P value, while the highest positional selectivity was found for the solvents with the lowest Log P values.

The tolerance of lipase to different reaction media and the use of solvent engineering make that the BCL-based biocatalysts have a high potential for synthesis reactions.

3. Synthesis of drugs

The synthesis of drugs and/or drug precursors using BCL has been important in the last ten years. This application, together with the enantiomeric resolution of compounds, are really

promising areas for this biocatalyst. The application of BCL in such reactions will be reviewed in this section.

The synthesis of enantiomerically pure compounds has become increasingly important because the beneficial effects of many drugs are given by only one of the enantiomers. Biocatalyzed reactions have been used for the synthesis of enantiomerically pure drugs. The biologically catalyzed reactions are environmentally friendly and allow the development of highly interesting processes from a commercial perspective.

The chemo- and regioselective acylation of amino alcohols, carbohydrates and nucleosides uses oxime esters as intermediates of importance (Fernández et al., 1991; Gotor and Pulido, 1991; Gotor and Morís, 1992; Pulido et al., 1992; Moris and Gotor, 1993; Pulido and Gotor, 1993). These nucleosides thus obtained play an important role in medicine (Isono, 1988) because they have antiviral and antineoplastic activity (MacCoss and Robins, 1990, Robins and Kini, 1990; Robins and Revankar, 1988). Traditional methods for the synthesis of oximes are complex or include the use of corrosive and/or flammable liquids (Patai and Rappoport, 1983; Houben et al., 1968). The focus of current studies is on finding more appropriate alternative methods for the synthesis of these compounds. With this purpose, oxime esters were produced by acylating aldoximes and ketoximes with vinyl acetate catalyzed by Lipase PS-C and Lipase PS-D (Fig. 2 and Table 1). The results showed that the conversion of oximes occurred only when the biocatalyst was present, and aliphatic oximes reacted faster than aromatic oximes. Polar solvents were the best reaction media. Tetrahydrofuran and 1,4-dioxane were the optimum solvents for lipase PS-C and lipase PS-D, respectively. Under the best reaction conditions, 50 mg of immobilized lipase catalyzed the acylation of 1 mmol of substrate (Salunkhe and Nair, 2000).

Insert Figure 2

Insert Table 1

Esters of L-ascorbic acid (vitamin C) are antioxidants widely used in food, pharmaceutical and cosmetic products. Conventionally, 6-O-palmitoyl-L-ascorbic acid is obtained by acylation of ascorbic acid with acid catalysts. This traditional chemical pathway leads to the formation of by-products that hinder the separation and purification of the desired product. In addition, the aforementioned synthesis pathway involves high energy consumption. Given the above difficulties, the acylation of ascorbic acid was carried out enzymatically as an alternative method. BCL was used for the synthesis of this antioxidant (Hsieh et al., 2006). In order to improve the enzyme activity, the lipase was coated with different nonionic surfactants. The biocatalyst was used in the synthesis of ascorbyl palmitate in an organic medium. The influence of different factors on the desired product yield was studied. Under optimal conditions (surfactant = propylene glycol monostearate, solvent = tert-butanol, temperature = 50 °C, molar ratio of ascorbic acid to palmitic acid = 1:6), a conversion of 47% was achieved after 24 hours of reaction. Only 6% conversion was reached using untreated lipase.

Fluorine has beneficial effects on the physiological activity and absorption of compounds containing it. Natural organic compounds rarely contain fluorine; however, a large proportion of the drugs sold in more recent years contains it (Thayer et al., 2006). β-amino acids together with their precursors and intermediates are extremely important in the production of medically interesting compounds (Juaristi and Soloshonok, 2005; Palomo et al. 2005). β-lactams (2-azetidinones) are important precursors in the synthesis of β-amino acids. By changing these compounds, replacing one or more hydrogens of the β-lactam skeleton by isosteric fluorine can increase the medical value of these products.

Li and Kanerva (2007) performed the synthesis of enantiomers of β-lactams (fluorinated and non-fluorinated) with a chemical-enzymatic approach. The enantiomers of 4-phenyl-2-azetidinones were obtained by a method that includes the lipase as a source of enantiomeric purity. Since residues in β amino acids are generally natural products found as β-amino amides or β-peptides, the authors studied the synthesis of β-dipeptides using β-lactams as acyl donors

to N-nucleophiles. They used fluoride to activate the ring of β-lactams. Tests were also performed using N-Boc-activated β-lactam (the tert-butyloxycarbonyl protecting group or BOC group is a protecting group used in organic synthesis). Fluorine favored the enantioselective synthesis of β-amino amides and β-dipeptides catalyzed by Lipase PS-D. Furthermore, the N-Boc activation promoted the chemical ring opening. This process was enhanced by the presence of lipase (Li et al., 2008).

One dipeptide that has received particular attention is β-alanyl-α-histidine, an α,β-dipeptide commonly known as carnosine (Fig. 3a). It is present in different structures of the human body and it has numerous beneficial properties (Dukic-Stefanovic et al., 2001; Pegova et al., 2000; Boldyrev and Abe, 1999). For these reasons, the interest in the synthesis of carnosine derivatives with therapeutic properties but with greater biological stability has increased (Cacciatore et al., 2005). D'Arrigo et al. (2009) studied the synthesis of analogs of carnosine by forming a peptide bond between a β-lactam and an alpha-amino protected acid. The reaction was catalyzed by Lipase PS-D (Fig. 3b).

Insert Figure 3

Among the wide variety of drugs with antitumor effects, floxuridine (5-fluoro-2'-deoxyuridine, FUdR) has been used for over 40 years in the treatment of colon carcinoma and liver metastases (Kemeny et al., 1999). Like other nucleoside analogues, this drug has poor oral bioavailability due to low cell penetrability. The synthesis of chemically modified derivatives has been used to overcome this problem. Li et al. (2007) conducted the regioselective caproylation of FUdR using Lipase PS-C as catalyst under mild reaction conditions to obtain 3'-O-caproyl-floxuridine. FUdR has two hydroxyl groups with similar reactivity, therefore it is extremely difficult to perform the regioselective acylation by the conventional chemical methodology. By using BCL in acetone or acetonitrile as the reaction solvent, 99% conversion and 93.5% of regioselectivity were achieved after 4 hours of reaction (Table 2). The biocatalyst

remained active for four batches. In a later report, Li et al. (2009) studied the synthesis of esters of FUdR again, but replacing the acyl donor vinyl caproate by arylaliphatic vinyl acid esters. Lipase PS-C was a good catalyst for the synthesis of 3-arylaliphatic acid esters of FUdR (Table 2). The authors evaluated the lipase selectivity towards various acyl donors, and they verified an increase in 3-regioselectivity with increased acyl chain length. Zhao et al. (2009) also studied the regioselective acylation of FUdR with vinyl crotonate as the acyl donor and catalyzed by Lipase PS-C. A simple and environmentally friendly process allowed to obtain 3'-O-crotonylfloxuridine with a high yield under mild reaction conditions. Different reaction parameters were optimized, and the results obtained under these conditions are shown in Table 2.

Another analog of deoxynucleoside with pharmacological applications is trifluridine (TFT). This drug is indicated for the treatment of primary keratoconjunctivitis and epithelial keratitis (Hobden et al., 2011; Skevaki et al., 2011; De Clercq, 2011). However, TFT presents the same problems as FUdR and other nucleoside drugs. As an alternative, Wang et al. (2011a) proposed the enzymatic synthesis of 3'-O-Acyl-trifluridines. This prodrug was obtained using Lipase PS-D to catalyze the acylation of TFT with different vinyl esters. The effect of various solvents on the activity and regioselectivity of the lipase was evaluated. Tetrahydrofuran (THF) was selected as the most appropriate solvent. The results obtained under optimal reaction conditions are presented in Table 2. They show that the regioselectivity of the lipase was independent of the acyl chain length.

6-Azauridine (AzUrd) is another nucleoside analog used for therapeutic purposes (Zeng et al., 2004) and it was regioselectively acylated using Lipase PS-D to obtain 3'-O-acyl-azauridine (Wang et al., 2012a; Wang et al., 2012b). This process was considerably easier compared to the traditional chemical pathway that requires multiple steps of protection and deprotection (Pejanović et al., 2006). Different reaction conditions were evaluated. The biocatalyst exhibited

high regioselectivity, and the results obtained under optimal experimental conditions are presented in Table 2.

Insert Table 2

A diterpene lactone (Pelletier et al., 1968) commonly known as andrographolide is the main compound extracted from a medicinal plant widely cultivated in South Asia (*Andrographis paniculata*). Acylated derivatives of andrographolide were studied as antitumor agents, achieving good activity against various types of cancer cells (Jada et al., 2007; Nanduri et al., 2004). However, regioselective acylation is difficult to achieve by a conventional chemical method because this compound has hydroxyl groups of similar reactivity (Jada et al., 2007). Regioselective acylation of this diterpene lactone was catalyzed by Lipase PS-C, with vinyl acetate as the acyl donor and acetone as the reaction solvent (Chen et al., 2010). Only 14-acetylandrographolide was generated (Fig. 4). The reaction was carried out for 4 hours at 50 °C and 0.11 water activity; under these conditions, 99% conversion was achieved. The authors also reported a high stability of the biocatalyst, maintaining a high percentage of its initial activity after eight successive uses.

Insert Figure 4

The pharmacological activity of some drugs resides in one of their enantiomers. That is also the case with pregabalin ((S)-3-(aminomethyl) -5-methylhexanoic), whose activity resides in the S-enantiomer. It is widely used in the treatment of peripheral neuropathic pain in adults, generalized anxiety disorder, fibromyalgia, and epilepsy, among other disorders (Sweetman, 2009). In order to obtain enantiomerically pure S-pregabalin, Zheng et al. (2012) carried out enantioselective hydrolysis of (S)-3-cyano-5-methylhexanoic acid ethyl ester catalyzed by BCL. The reaction was conducted with high substrate concentrations at 35 °C and pH 6.0. This lipase had a good performance as catalyst in the synthesis of pregabalin, obtaining good yields and

high enantiomeric purity (44.5% and 99.2%, respectively). The authors did not evaluate the reuse of the biocatalyst, which would reduce process costs.

Improvements in the immobilization processes to avoid enzyme leaching and biocatalyst reuse are two key aspects for the development of large-scale processes with competitive costs.

4. Synthesis and degradation of polymers

The synthesis of new materials is rarely performed using lipases, but the versatility of these enzymes has allowed their use in the synthesis or degradation of materials, particularly polymers.

Polymer modification enables their use as catalysts or substrates in the field of organic synthesis, as adsorbents with specific properties, or makes them biodegradable. These modifications may be carried out through the incorporation of sugars to traditional polymers, such as polyesters, polyamides, polyacrylates, etc. (Sherrington and Hodge, 1988; Kondo, 1987; Murphy et al., 1988; Andrade, 1976; Swift Glass and G, 1989; Selegny, 1979; Allcock and Scopelianos, 1983; Allcock and Pucher, 1991). However, functionalization with sugars is extremely complex because they contain multiple hydroxyl groups. Achieving a unique and selective binding between the polymer and the sugar is difficult (Haines, 1981). Martin et al. (1992) developed a chemo-enzymatic method for preparing functionalized polyacrylates with different monosaccharides. 6-Acryloyl esters were obtained by transesterification of monosaccharides with vinyl acrylates catalyzed by BCL and using pyridine as the reaction solvent. After obtaining the vinyl esters, the authors performed the polymerization to obtain the polyacrylates as products. This method allowed the synthesis of poly(methyl 6-acryloyl-β-galactoside) with good yields.

Some polymers are usually mixed with other compounds to improve the characteristics or quality thereof (Liu et al., 2009; Maafi et al., 2010). One example is poly-ε-caprolactone (PCL). PCL is a biodegradable polymer that is hydrolyzed to obtain smaller units with specific properties. Traditionally, PCL hydrolysis is carried out with a chemically-catalyzed process using

free radical as initiators (Li et al., 1997). This process is complex, expensive and time-consuming. PCL hydrolysis catalyzed by BCL was studied by Chew et al. (2015). The authors performed the extractive bioconversion of the polymer to its monomers and oligomers in an aqueous two-phase system (ATPS).

On the other hand, the development of biodegradable polymers has emerged as a palliative to the environmental problems caused by plastic waste. The most important biodegradable polymers developed to date are aliphatic polyesters such as PCL, poly-*L*-lactic acid, poly-3-hydroxybutyrate and polybutylene succinate (PBS) (Decker and Bendaikha, 1998; Weiss, 1962; Anderson et al., 1998). Taniguchi et al. (2002) studied the enzymatic hydrolysis of PBS and polybutylene succinate-co-L-lactate (PBSL) catalyzed by BCL. They found that fibers of PBSL were easily hydrolyzed by the lipase, whereas fibers of PBS suffered little hydrolysis. In addition, the enzymatic hydrolysis of films of both polymers was largely achieved. These results suggest that this reaction depends on the crystallinity of the polymer and the fiber orientation. Along the same lines, Honda et al. (2003) investigated the hydrolysis of polybutylenesuccinate-co-terephthalate (PBST) with BCL. They reported an increase in the degradability of PBST with increasing butylenesuccinate (BS) content. An overall mechanism of the enzymatic hydrolysis of this copolyester was proposed in the same work.

The studies on the handling of polymers using this lipase are scarce. Compete with low-cost polymers obtained from traditional and well-installed technologies is probably the biggest challenge of this field of study.

5. Synthesis and modification of glycerides.

Acylglycerides (AG) are defined as esters of glycerol (1,2,3-propanetriol) with fatty acids (FA). Depending on the number of esterified fatty acids on the glycerol backbone, monoglycerides (MAG), diglycerides (DAG) or triglycerides (TAG) are obtained.

MAGs are nonionic surfactants used in pharmaceutical, food, and cosmetic industries. The glycerolysis reaction of triolein catalyzed by BCL was studied in a solid-phase system in order to obtain monoglycerides (Bornscheuer and Yamane, 1994). The reaction catalyzed by crude lipase was carried out for 100 h and 73% of MAG was obtained. Lipase maintained 15% of its initial activity. When the reaction was catalyzed with the purified lipase, a significant deactivation was verified and only 11% of MAG was generated. Finally, BCL was deposited on celite (remains of tiny aquatic plants called diatoms). In this case, a substantial improvement in stability after 100 h of reaction was achieved and MAG concentration exceeded 87%.

The glycerolysis reaction to generate MAGs was also studied by de Freitas et al. (2010), who used babassu oil as substrate. The reaction was conducted in a continuous packed-bed reactor at 50 °C and with 15:1 molar ratio of glycerol to oil. The catalyst was BCL immobilized on a hybrid support based on polysiloxane-polyvinyl alcohol (SiO_2-PVA) following a method previously developed by the authors (Santos et al., 2008). The reactor was operated continuously for 22 days. The concentration of MAGs had values between 25 and 33% (Table 3).

Insert Table 3

Bornscheuer et al. (1994b) studied the esterification of glycerol with fatty acids or with the corresponding vinyl ester. Different strategies for the synthesis of MAGs were tested: a) the esterification of glycerol with lauric acid in a bis-(2-ethylhexyl)sulfosuccinate sodium salt/isooctane microemulsion system, b) the transesterification of glycerol with vinyl laurate in a system with or without solvent, c) the glycerolysis of trilarin, and d) the transesterification of 1,2-O-isopropylidene glycerol with vinyl laurate in different reaction media. The authors reported the exclusive formation of MAGs in the latter cases (c and d), whereas mono- and diglycerides were detected in the other cases.

The selectivity of BCL toward fatty acids of different chain length during the synthesis of acylglycerides has been studied by several authors (Chang et al., 1999; Lee and Parkin, 2003; Fu and Parkin, 2004). In their report, Chang et al. (1999) evaluated the selectivity of BCL in the esterification reaction of glycerol (and glycerol analogues) with fatty acids of a chain length in the range C4 to C16 (Table 4). The selectivity towards different fatty acids was influenced by the alcohol used. The functional group located in the sn-2 position alcohol was key in the selectivity of the lipase. FA selectivity was evaluated by a competitive factor (α value), which is proportional to the specificity constant, V_{max}/K_M (Deleuze et al., 1987 and Rangheard et al., 1989). In the selectivity studies reviewed here, C8 was taken as the reference substrate and assigned an α-value of 1. The greater the α value, the greater the selectivity (V_{max}/K_M) toward a particular FA substrate.

Insert Table 4

A report submitted by Lee and Parkin (2003) confirms the information presented above. They evaluated the selectivity of the lipase toward fatty acids in different multicompetitive reactions. The greatest competitive factor values were obtained for C8, C16, and C18:2. Finally, in another study on the selective incorporation of fatty acids by Fu and Parkin (2004), the esterification of glycerol was performed with a multicompetitive mixture of saturated fatty acids and oleic acid (C18:1). The selective incorporation of fatty acids into MAG, DAG and TAG was evaluated. Their results are shown in Table 5.

Insert Table 5

The use of DAGs as nonionic emulsifiers in foods, cosmetics and pharmaceuticals is well known (Gunstone, 1999; Fureby et al., 1997). DAGs are also a natural minor component of various edible oils (D'alonzo et al., 1982).

Bi et al. (2015) studied the effects caused by different solvents on the positional selectivity of Lipase PS-C for the esterification of glycerol with oleic acid to obtain DAG. The influence of

the solvents was analyzed according to their values of log P. The authors reported an increase in the DAG yield associated with the increase in the value of log P. The yield of diglyceride in acetone while the highest yield of DAG corresponded to n-octane. The results for all the studied solvents are presented in Table 6. They indicate that the lipase was more active in the solvents with higher log P values. This could be attributed to the fact that the solvents with high log P can retain the water present around the active site of the lipase, which is essential for enzyme activity. The values of 1,3-diolein/1,2-diolein ratio were also affected by the reaction solvent. However, in this case, the solvent effect was the opposite to that reported for the enzyme activity. The greatest value of the 1,3-DAG to 1.2-DAG ratio was obtained in acetone. The ratio of 1.3-diolein to 1.2-diolein decreased with increasing log P values (Table 6). The secondary structure of the lipase was apparently influenced by the solvent, affecting the coordination of the substrates with the active site, and thus selectivity.

Insert Table 6

TAGs are the major components of oils and fats. Structured triacylglycerols (ST) are any fats that are modified or restructured from natural oils and fats, or fatty acids therefrom, having special functionality or nutritional properties for edible or pharmaceutical purposes (Høy and Xu, 2001). In our extensive review, only one report was found in which the synthesis of ST was performed using BCL (Wongsakul et al., 2004). In this work, sn-2 position of 1,3-dilaurin (or 1,3-dicaprylin) was esterified with oleic acid vinyl ester using BCL as catalyst. Reactions were carried out in a system with or without solvent at 60 °C, and using different technologies to control water in the reaction medium. Thus the desired triglycerides (1,3-dicapryloyl-2-oleyl-glycerol or 1,3-dilauroyl-2-oleyl-glycerol (CyOCy or LaOLa)) were obtained with yields of 87 and 78%, respectively.

The use of BCL in the synthesis of different glycerides is relatively scarce compared to other lipases. This biocatalyst has high potential in this area. The synthesis of monoglycerides,

diglycerides, and structured triglycerides could be explored with BCL because of their tolerance to various reaction conditions, and the ability to modulate its selectivity with the use of solvents.

6. Synthesis of biodiesel

Biodiesel is defined as a monoalkyl fatty acid ester (preferentially methyl and ethyl esters) (Knothe, 2001). It is an alternative diesel fuel because of its environmental benefits such as being biodegradable, nontoxic and having a low carbon dioxide emission profile in an overall balance (Ma and Hanna, 1999). Chemical transesterification to obtain it can be complex if the feedstock contains high free fatty acids levels or water. Enzymatic approaches can overcome these problems because lipases can operate under a variety of conditions in the synthesis of alkyl esters (Shimada et al., 1999; Hsu et al., 2002; Nelson et al., 1996). BCL was tested for its robustness towards methanol in transesterification assays. The results showed that BCL retained its native structure and activity upon prolonged incubations at high methanol concentrations, an interesting feature compared to other lipases, which are known to be inhibited by methanol (Fjerbaek et al., 2009; Lotti et al., 2015; Chen and Wu, 2003; Shimada et al., 2002; Salis et al., 2005). BCL has an attractive potential for use as biocatalyst for biofuel production.

6.1. Biodiesel from conventional oils

The synthesis of biodiesel using edible oils as raw material and BCL as catalyst was reported in several works. Noureddini et al. (2005) studied the enzymatic transesterification of soybean oil with methanol and ethanol. The lipase was previously immobilized by entrapment in a hydrophobic sol-gel matrix. The transesterification of 10 g of oil was catalyzed with 475 mg of biocatalyst at 35 °C. When methanol was used as the alcohol, the reaction was carried out with 1:7.5 oil/alcohol molar ratio and 500 mg of water, whereas when ethanol was used, the

oil/alcohol molar ratio was 1:15.2 and 300 mg of water were added. The results obtained under these conditions are shown in Table 7. The immobilized lipase was more active than its free form, it also had greater stability and could be reused without significant changes in activity.

Jegannathan et al. (2010) studied the enzymatic transesterification of palm oil with methanol catalyzed with BCL encapsulated within κ-carrageenan (a biopolymer). The reaction conditions were optimized and the transesterification of 10 g of oil was carried out at 30 °C using 5.25 g of biocatalyst, 1 g of water, and 1:7 oil/methanol molar ratio. The reaction time was 72 hours. The results obtained under these conditions are shown in Table 7. The biocatalyst obtained was active and stable, and after 5 cycles it maintained more than 82% of its initial activity.

Li et al. (2011b) studied the transesterification of soybean oil with methanol catalyzed by BCL covalently immobilized on polyacrylonitrile nanofibrous membranes, which were previously activated by amidation. The reaction conditions were optimized, and the results are presented in Table 7. The biocatalyst retained 91% of its initial conversion capacity after 10 successive uses.

The synthesis of biodiesel by the transesterification of soybean oil catalyzed with BCL was also studied by Andrade et al. (2016). In their report, the lipase was covalently immobilized on magnetic nanoparticles precoated with a thin layer of polydopamine. The reaction was carried out at 37 °C, and 90% yield was achieved. However, the biocatalyst only showed good activity for three successive uses. Along the same lines, Wang et al. (2011b) had previously designed a packed-bed reactor system using lipase immobilized on Fe_3O_4 nanoparticles as catalyst. The methanolysis of soybean oil was carried out in a single packed-bed reactor, and after 240 h of continuous operation the conversion remained as high as 45% at 0.25 mL/min. Using a system of four reactors, the results improved considerably. The conversion remained around 88% for the first 182 h, and decreased to 75% after 240 h.

Insert Table 7

6.2. Biodiesel from unconventional oils

The use of new sources of oil for biodiesel production is presented as an alternative to avoid using food for fuel generation. *Madhuca indica* (mahua, mahwa or Iluppai) is a tree native to the tropical forests of India. The high content of free fatty acids present in the Mahua fat makes transesterification by the conventional chemical method difficult (Ghadge and Raheman, 2005). Kumari et al. (2007) proposed the use of BCL immobilized in different forms to obtain ethyl esters from the fat of Mahua. The biocatalysts tested were: lipase immobilized on accurel (a polypropylene membrane), cross-linked enzyme aggregates (CLEAs) and protein-coated microcrystals (PCMCs). The results for each catalyst are presented in Table 8.

Freitas et al. (2009) studied the synthesis of biodiesel by the ethanolysis of babassu oil. This oil is extracted from the seeds of the babassu palm (Orbignya phalerata and Orbignya oleifera. The transesterification reaction was conducted for 48 h at 39 °C using an oil/ethanol molar ratio of 1:7. BCL immobilized on a hybrid polymer based on polysiloxane–polyvinyl alcohol (silica-PVA) was used as catalyst. The results are presented in Table 8.

Da Rós and coauthors also analyzed the synthesis of biodiesel from babassu oil (Da Rós et al., 2010). In their work, the ethanolysis of Babassu oil was mediated by two different biocatalysts: BCL was covalently immobilized on niobium oxide (Nb_2O_5) and silica-PVA. The transesterification reaction was carried out at 50 °C using 12 g of substrate in an oil/alcohol molar ratio of 1:12. The reaction time was 48 h and 2.4 g of biocatalyst was added. The obtained results are shown in Table 8. BCL immobilized on silica-PVA was also used in the ethanolysis of babassu oil carried out in a packed-bed reactor operated continuously (Simões et al., 2015). High yield values were obtained, and the properties of the biocatalyst remained virtually unchanged for 32 days.

Jatropha oil has emerged as another alternative for biofuel production without resorting to food oils. Shah and Gupta (2005) carried out biodiesel synthesis by ethanolysis from this oil.

They studied the effect of several parameters for different biocatalysts. The best results were obtained when the ethanolysis was carried out for 8 h at 50 °C using BCL immobilized on celite in a system containing 4-5 wt.% of water (Table 8). The ethanolysis of jatropha oil was also studied by Soumanou et al. (2012). The lipase was immobilized by adsorption on polypropylene macroporous (Accurel 1282) and the reaction was conducted for 16 h using 3 wt.% of biocatalyst (Table 8). The lipase activity remained virtually unchanged after 10 successive batches. Abdulla and Ravindra (2013a) developed a catalyst to carry out the ethanolysis of Jatropha oil. BCL was first cross-linked with glutaraldehyde followed by entrapment in a hybrid matrix (alginate and k-carrageenan). A biodiesel yield of 100% was achieved by performing the ethanolysis of 10 g of oil using 5.25 g of biocatalyst, an oil/alcohol molar ratio of 1:10, 1 g of water, at 35 °C (Table 8).

The synthesis of biodiesel from Jatropha oil was also examined by Kawakami et al. (2011). In this case, methanolysis of an oil containing 18% of free fatty acids was carried out. BCL was immobilized on an n-butyl-substituted silica monolith. This biocatalyst was used in the reaction, which was performed with two different configurations. First, the methanolysis was conducted in a batch system at 40 °C with a mixture of oil and alcohol at a stoichiometric ratio and adding 0.6 wt.% of water. The results are shown in Table 8. Then, the reaction was performed in a continuous system using 1.67 g of BCL immobilized on a silica monolith, with a flow rate of 0.6 mL/h (Table 8). A high percentage of the initial activity was maintained after 50 days of operation. You et al. (2013) also studied the methanolysis of this oil. They used BCL immobilized on attapulgite modified by cross-linking reaction as catalyst. Different factors were evaluated using an experimental design. The best conditions were obtained when the reaction was carried out as follows: 10 g jatropha oil, 2.4 g methanol, 7 wt.% water, 10 wt.% immobilized lipase, temperature 35 °C, and time 24 h. The results for these conditions are presented in Table 8.

Insert Table 8

6.3. Biodiesel from waste cooking oils

Obtaining biodiesel by the conventional catalytic route using low-cost unrefined oils or waste cooking oils as raw materials has been complex due to the high concentrations of fatty acids and/or water (Nelson et al., 1996). For this reason, there is an increasing interest in the development of alternative technologies for producing biodiesel from these feedstocks.

Alcoholysis of restaurant grease was catalyzed by immobilized BCL within a phyllosilicate sol-gel matrix (Hsu et al., 2002). The reactions were carried out for 24 h at 40 °C and using 1.74 g of grease, 8 mmol of alcohol and 10 wt.% of biocatalyst (based on grease). Under these conditions, the biodiesel yield values were of 88%, 88%, 86%, 87%, 46%, 97% and 72% using methanol, ethanol, 95% ethanol, propanol, isopropanol, butanol, and isobutanol as alcohol, respectively. In a subsequent study by the same authors (Hsu et al., 2004), the aforementioned biocatalyst was used in a packed-column system with recirculation. Ethanol and restaurant grease were used as substrate. Different parameters were evaluated to maximize production of ethyl esters: temperature (40-60 °C), flow rate (5-50 mL/min), and time (8-48 h). The optimal conditions were: flow rate 30 mL/min; temperature 50 °C; mole ratio of substrates 4:1 ethanol/grease; reaction time 48 h. The biocatalyst was active and stable during the continuous process, and ethyl ester yields were higher than 96%.

The use of BCL as a catalyst for the synthesis of biodiesel is promising, particularly for those oils that can not be processed by the conventional catalytic route. The costs associated with the biocatalyst make the bioprocess uncompetitive, however great effort is being made to increase the stability of the lipase to reduce costs.

7. Conclusions

BCL is a lipase used in a variety of reactions, such as esterification (Priya and Chadha, 2003), acylation (Hsieh et al., 2006), hydrolysis (Zheng et al., 2012) and transesterification (Martin et al., 1992), among others.

The main advantage of this lipase is its heat resistance and tolerance to a large number of solvents and short-chain alcohols (Bornscheuer et al., 1994). Due to these characteristics, this lipase is particularly interesting to conduct esterification and transesterification reactions or reactions in solvent systems.

BCL shows no special positional specificity and a high hydrolyzing activity regardless of the length of the fatty acid chain (Kim et al., 1992). However, in esterification reactions BCL exhibits a preference for sn-1 and sn-3 positions in the presence of hydrophilic solvents (Bi et al., 2015) and a marked selectivity toward C8, C10 and C18:2 fatty acids (Chang et al, 1999; Lee and Parkin, 2003).

This lipase has been used in free or immobilized form in the synthesis of various products (Chen et al., 2010; Soumanou et al., 2012). The use of immobilized BCL allows for better operating conditions and an easier recovery of the biocatalyst (Pencreac'h and Baratti, 1997; Hsu et al., 2000). The modification of the microenvironment by bioimprinting or interfacial activation is extremely interesting to increase enzyme activity (Liu et al., 2011a). Nevertheless the problem of BCL desorption from supports needs to be solved.

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Captions for figures

Figure 1. Structure of open form of BCL. The catalytic triad in the active site is shown in a red stick representation (Liu et al., 2015).

Figure 2. Lipases PS-C and PS-D catalyzed reaction of oximes (Salunkhe and Nair, 2000).

Figure 3. a) Structure of the dipeptide carnosine. b) Mechanism of lipase-catalysed dipeptide formation (D'Arrigo et al., 2009).

Figure 4. Lipase-catalyzed regioselective acylation of andrographolide with vinyl acetate in acetone (Chen et al., 2010).

Captions for tables

Table 1. Lipases PS-C and PS-D catalyzed reaction of oximes (Salunkhe and Nair, 2000).

Table 2. Acylation of nucleosides catalyzed by Burkholderia cepacia lipase

Table 3. Glyceride profile in the continuous glycerolysis of babassu oil using BCL immobilized on SiO_2–PVA under inert atmosphere (de Freitas et al., 2010).

Table 4. α values for the series of n-fatty acid substrates with different alcohols in reactions mediated by BCL (Chang et al., 1999).

Table 5. Relative selectivity constants (α-values) for FA incorporation into specific acylglycerol pools using BCL (Fu and Parkin, 2004).

Table 6. Solvent effect on the enzymatic 1,3-diolein synthesis catalyzed by BCL (Bi et al., 2015).

Table 7. Biodiesel synthesis using conventional oils catalyzed by BCL.

Table 8. Biodiesel synthesis using unconventional oils catalyzed by BCL.

Table 1. Lipases PS-C and PS-D catalyzed reaction of oximes (Salunkhe and Nair, 2000).

Reactant	R1	R2	Product	Time (h)	Yield in I (%)	Yield in II (%)
1a	CH_3	CH_3	2a	7	93	92
1b	CH_3	CH_2CH_5	2b	7	94	91
1c	$(CH_2)_5$		2c	7	93	93
1d	C_6H_5	H	2d	9	92	91
1e	C_6H_5	CH_3	2e	9	92	93
1f	P-$H_3C.C_6H_4$	CH_3	2f	11	89	92
1g	P-$O_2N.C_6H_4$	CH_3	2g	8	94	95
1h	C_6H_5	C_6H_5	2h	9	95	96

Table 2. Acylation of nucleosides catalyzed by Burkholderia cepacia lipase

Nucleoside	Acyl donor	Solvent	Temperature (°C)	Time (h)	Conversion (%)	3'-Regioselectivity (%)	Reference
FUdR	Vinyl caproate	Acetone	35	4	100	93.5	Li et al. (2007)
FUdR	Vinyl benzoate	THF	50	82.5	98	85	Li et al. (2009)
FUdR	Vinyl phenylacetate	THF	50	7	99	90	Li et al. (2009)
FUdR	Vinyl 3-phenylpropionate	THF	50	4	99	93	Li et al. (2009)
FUdR	Vinyl 4-phenylbutyrate	THF	50	2	99	99	Li et al. (2009)
FUdR	Vinyl 5-phenylvalerate	THF	50	6	99	99	Li et al. (2009)
FUdR	Vinyl crotonate	1,4-dioxan	40	50	99	85	Zhao et al. (2009)
TFT	Vinyl hexanoate	THF	50	-	99	99	Wang et al. (2011)a
TFT	Vinyl decanoate	THF	50	-	99	99	Wang et al. (2011)a
TFT	Vinyl myristate	THF	50	-	99	99	Wang et al. (2011)a
AzUrd	Vinyl laurate	Acetone	40	8	99	74	Wang et al. (2012)a
AzUrd	Vinyl myristate	Acetone	40	8	98	75	Wang et al. (2012)a
AzUrd	Vinyl palmitate	Acetone	40	8.5	98	77	Wang et al. (2012)a
AzUrd	Vinyl stearate	Acetone	45	5	99	86	Wang et al. (2012)b

Table 3. Glyceride profile in the continuous glycerolysis of babassu oil using BCL immobilized on SiO_2–PVA under inert atmosphere (de Freitas et al., 2010).

Time (days)	MAG (%)[a]	DAG (%)[a]	TAG (%)[a]	Time (days)	MAG (%)[a]	DAG (%)[a]	TAG (%)[a]
1	12	63	25	12	27	73	0
2	31	54	15	13	25	75	0
3	33	66	1	14	24	76	0
4	32	65	3	15	25	75	0
5	31	67	2	16	25	75	0
6	32	67	1	17	25	75	0
7	28	72	0.5	18	26	74	0
8	26	74	0	19	26	74	0
9	25	75	0	20	27	73	0
10	22	78	0	21	26	74	0
11	25	75	0	22	26	74	0

a Molar percentage.

Table 4. α values for the series of n-fatty acid substrates with different alcohols in reactions mediated by BCL (Chang et al., 1999).

Serial Number	Acyl carbon length	Alcohol acceptor	Competitive Factors (α values)	Serial Number	Acyl carbon length	Alcohol acceptor	Competitive Factors (α values)
1	4	n-Pro	0.25	15	4	1,2-PD	0.46
2	6	n-Pro	0.32	16	6	1,2-PD	0.55
3	8	n-Pro	1	17	8	1,2-PD	1
4	10	n-Pro	0.53	18	10	1,2-PD	0.42
5	12	n-Pro	0.43	19	12	1,2-PD	0.59
6	14	n-Pro	0.8	20	14	1,2-PD	0.81
7	16	n-Pro	1.27	21	16	1,2-PD	0.64
8	4	1,3-PD	0.25	22	4	Gly	0.7
9	6	1,3-PD	0.38	23	6	Gly	0.81
10	8	1,3-PD	1	24	8	Gly	1
11	10	1,3-PD	0.6	25	10	Gly	0.59
12	12	1,3-PD	0.46	26	12	Gly	0.32
13	14	1,3-PD	0.6	27	14	Gly	0.55
14	16	1,3-PD	0.7	28	16	Gly	0.39

n-Pro = n-Propanol, 1,3-PD = 1,3-propanediol, 1,2-PD = 1,2-propanediol and Gly = Glycerol

Table 5. Relative selectivity constants (α-values) for FA incorporation into specific acylglycerol pools using BCL (Fu and Parkin, 2004).

Fatty acid	Glycerides	Competitive factors (α values)
C4	MAG	0.13 ± 0.010
	DAG	0.11 ± 0.010
	TAG	0.02 ± 0.005
C6	MAG	0.42 ± 0.020
	DAG	0.43 ± 0.020
	TAG	0.39 ± 0.015
C8	MAG	1.00 ± 0.00
	DAG	1.00 ± 0.00
	TAG	1.00 ± 0.00
C10	MAG	1.20 ± 0.060
	DAG	1.30 ± 0.070
	TAG	0.80 ± 0.060
C12	MAG	0.60 ± 0.030
	DAG	0.67 ± 0.030
	TAG	0.71 ± 0.030
C14	MAG	0.72 ± 0.045
	DAG	0.75 ± 0.045
	TAG	0.73 ± 0.045
C16	MAG	1.15 ± 0.080
	DAG	1.06 ± 0.060
	TAG	1.05 ± 0.060
C18	MAG	0.66 ± 0.040
	DAG	0.62 ± 0.03
	TAG	0.64 ± 0.045
	MAG	0.17 ± 0.010

C18:1	DAG	0.36 ± 0.020
	TAG	0.58 ± 0.025

Table 6. Solvent effect on the enzymatic 1,3-diolein synthesis catalyzed by BCL (Bi et al., 2015).

Solvent	log P	Diolein yield (%)	1,3-Diolein/1,2-Diolein
Acetone	-0.23	58.8	31.1
Tetrahydrofuran	0.49	75.3	25.8
t-Butanol	0.8	81.1	22.6
4-Methyl-2-pentanone	1.31	82.5	17.8
Chloroform	2.0	85.0	12.1
Toluene	2.5	87.3	10.2
Tetrachloromethane	3.0	87.9	8.9
Cyclohexane	3.2	88.2	8.7
n-Hexane	3.5	88.3	8.1
n-Heptane	4.0	88.5	7.6
n-Octane	4.5	88.5	7.1

Table 7. Biodiesel synthesis using conventional oils catalyzed by BCL.

Oil source	Alcohol	Biocatalyst	Temperature (°C)	Time (h)	Oil Conversion (%)	Biodiesel yield (%)	Reaction medium	References
Soybean oil	Methanol	BCL-entrapped in hydrophobic sol-gel	35	1	100	67	Solvent free	Noureddini et al. (2005)
Soybean oil	Ethanol	BCL-entrapped in hydrophobic sol-gel	35	1	100	65	Solvent free	Noureddini et al. (2005)
Palm oil	Methanol	BCL-encapsulated within κ-carrageenan	30	72	100	100	Solvent free	Jegannathan et al. (2010)
Soybean oil	Methanol	BCL on polyacrylonitrile nanofibrous membrane	30	24	90	---	Solvent free	Li et al. (2011)
Soybean oil		BCL-polydopamine-Fe_3O_4	37	12	93	90	Solvent free	Andrade et al (2016)
Soybean oil	Methanol	BCL-Fe_3O_4 nanoparticle (continuous operation)	40	192	88	---	*n*-Hexane	Wang et al. (2011)b

Table 8. Biodiesel synthesis using unconventional oils catalyzed by BCL.

Oil source	Alcohol	Biocatalyst	Temperature (°C)	Time (h)	Oil Conversion (%)	Transesterification yield (%)	References
Madhuca indica	Ethanol	BCL-Accurel	40	6	96	---	Kumari et al. (2007)
Madhuca indica	Ethanol	BCL-CLEAs	40	2.5	92	---	Kumari et al. (2007)
Madhuca indica	Ethanol	BCL-PCMCs	40	16	99	---	Kumari et al. (2007)
Babassu oil	Ethanol	BCL-Sílica-PVA	39	48	---	98	Freitas et al. (2009)
Babassu oil	Ethanol	BCL-SiO_2-PVA	50	48	---	100	Da Rós et al. (2010)
Babassu oil	Ethanol	BCL-Nb_2O_5	50	48	---	74	Da Rós et al. (2010)
Babassu oil	Ethanol	BCL-SiO_2-PVA continuous mode	50	11	---	96	Simões et al. (2015)
Jatropha oil	Ethanol	BCL-celite	50	8	---	98	Shah et al. (2003)
Jatropha oil	Methanol	BCL-hydrophobic silica monolith	40	12	---	90	Kawakami et al. (2011)
Jatropha oil	Methanol	BCL-hydrophobic silica monolith continuous mode	40	---	---	95	Kawakami et al. (2011)
Jatropha oil	Ethanol	BCL-Accurel 1282	---	16	93	---	Soumanou et al. (2012)
Jatropha	Ethan	BCL-hybrid	35	24	100	100	Abdulla and

oil	ol	matrix					Ravindra (2013)
Jatropha oil	Methanol	BCL-attapulgite	35	24	---	94	You et al. (2013)

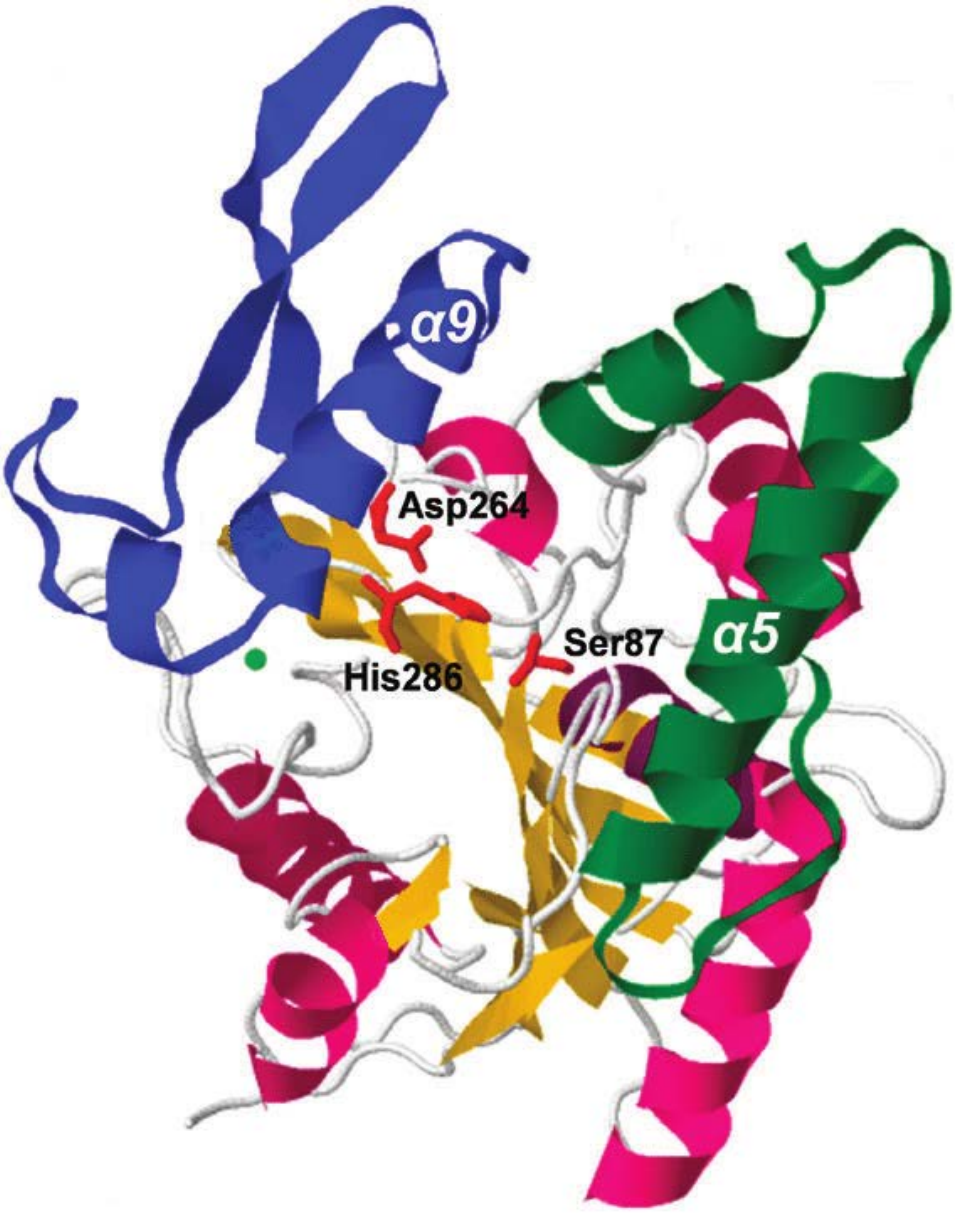

Figure 1

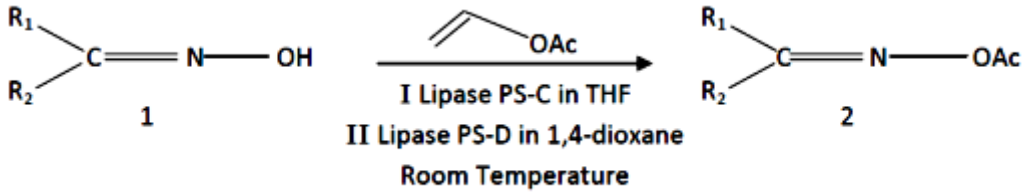

Figure 2

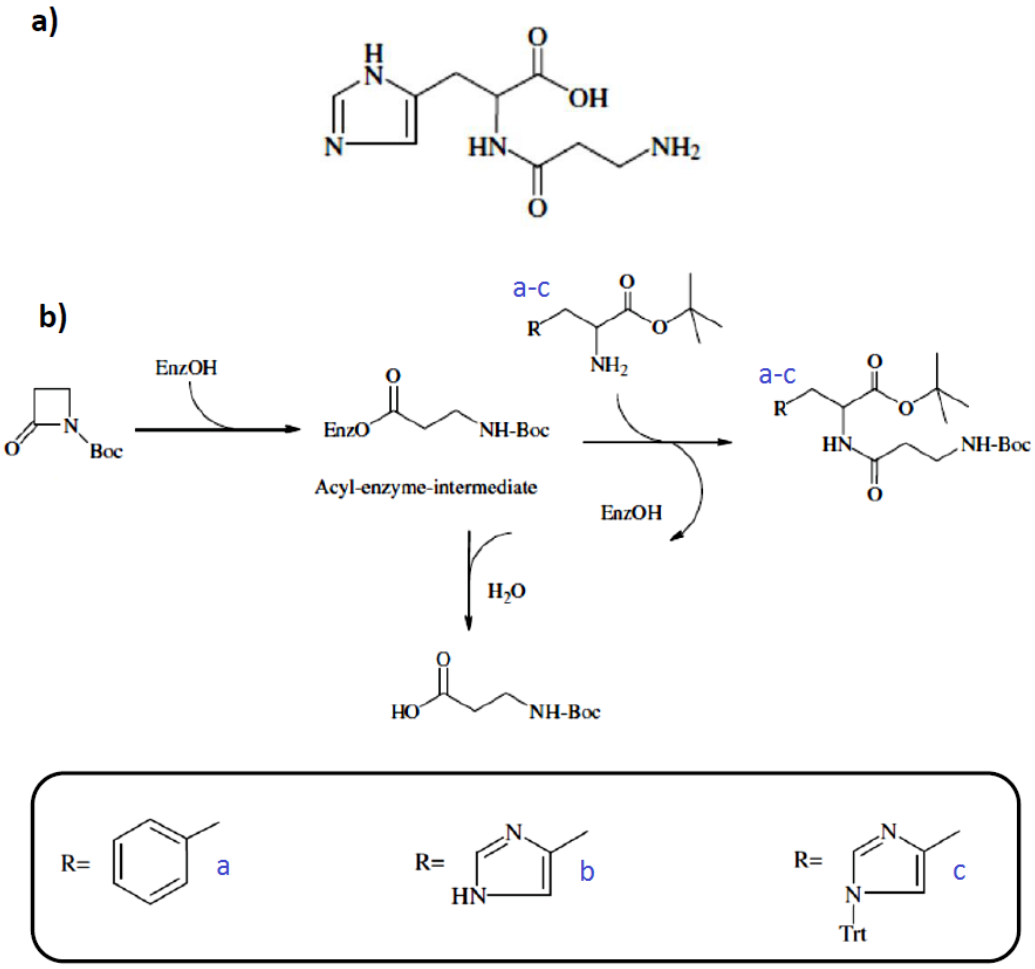

Figure 3

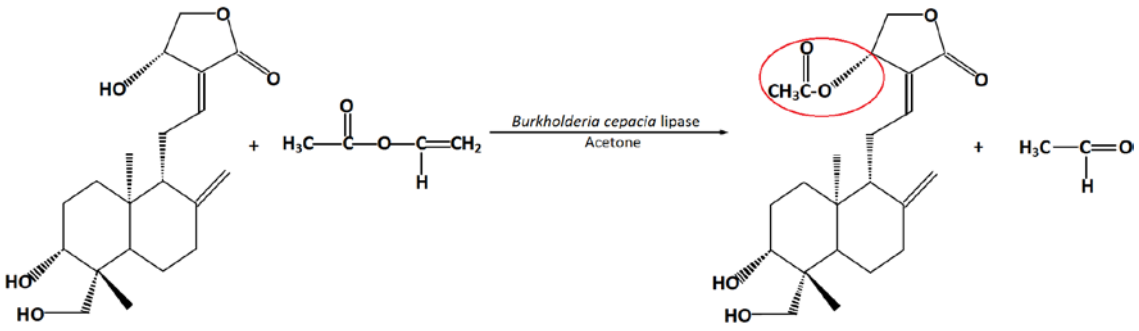

Figure 4