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Production and activity of extracellular lipase from *Luteibacter* sp.

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Abstract Microbial lipases are widely used in industrial applications due to their versatility, and the characterization of new lipase-producing microorganisms could provide new sources of these enzymes, with different specificities and better activities. In this context, we have improved lipase production by Luteibacter sp. by using basal medium supplemented with 2 % olive oil, a pH of 6 and a growth temperature of 37 °C. The enzyme extraction process with the addition of 0.25 % Tween 80 increased lipase activity. Implementation of these modifications increased lipase activity by approximately 430 %. The lipase activities produced in the culture supernatant (LCS) and extracted with Tween 80 (LCST80) were characterized. Both extracts hydrolyzed p-nitrophenyl (pNP) esters with different acyl chain lengths, with a preference for short acyl lengths, and had optimum activity at 45 °C. The LCS was stable at acidic and alkaline pH, but LCST80 was only stable at alkaline pH. Methanol, SDS, Triton X-100, EDTA, and EGTA did not affect lipase activity, while divalent cations (Ca²⁺, Zn²⁺, Mg^{2+}) - with the exception of Co^{2+} increased lipase activity. Both extracts showed transesterification activity on pNP ester substrates, and both were able to hydrolyze different natural lipids. The characterization of lipase

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produced by *Luteibacter* sp. introduces this recently described genus as a new source of lipases with great biotechnological potential.

Keywords Lipase · *Luteibacter* sp. · Transesterification · Biodiesel · Lipase extraction · Enzyme characterization

Introduction

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are serine hydrolases that catalyze hydrolysis of the acyl ester bonds of lipids at the oil/water interface (Sharma et al. 2001; Reetz 2002). Many lipases are active in organic solvents, where they promote reactions of synthesis and hydrolysis of ester bonds, and other reactions of industrial relevance (Sharma et al. 2001). In contrast to chemical methods, enzymatic reactions can usually be performed under mild conditions and at low cost.

Microbial enzymes are widely used in industrial applications because of their versatility, fast growth of microorganisms, and the easiness of genetic manipulation and culture (Hasan et al. 2006). Lipases do not usually require cofactors, and considering the wide variety of reactions that they catalyze, they are used in a variety of industrial applications, such as the leather and detergent industry, waste treatment, food processing, flavor development, cosmetics, pharmaceuticals, and biodiesel production (Sharma et al. 2001; Jaeger and Eggert 2002). Crude vegetable oils and animal fat and their industrial derivatives (soap stocks, industrial fatty acids, tallow) can be used as substrates for lipase production (Kamzolova et al. 2005; Papanikolaou et al. 2007).

Isolation of new lipase-secreting bacteria, studies aimed at increasing the production of these enzymes by varying culture conditions, and characterization of lipolytic activity

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are very important due to their potential to provide new lipase sources with different biochemical characteristics and a wide range of applications. Several authors describe the importance of the yeast Yarrowia lipolytica, which has been very well studied (Hadeball 1991; Pereira-Meirelles et al. 1997; Fickers et al. 2005; Bankar et al. 2009), and the filamentous fungus Aspergillus terreus (Sethi et al. 2013). Other researchers have studied genes encoding the extracellular lipases of Y. lipolytica (Pignede et al. 2000; Desfougères et al. 2010). Lipase production by Y. lipolytica has been shown to be stimulated by the presence of long-chain fatty acids, triacylglycerols and organic nitrogen, surfactants, aeration, and the agitation of the culture medium (Pereira-Meirelles et al. 2000; Dominguez et al. 2003; Alonso et al. 2005; Kamzolova et al. 2005). However, as only about 2 % of the world's microorganisms have been screened as enzyme sources (Hasan et al. 2006), it is clearly important ti study newly described genera of microorganisms under different culture conditions to determine their lipase-producing activity.

A Gram-negative bacterium, *Luteibacter* sp., which was isolated and described for the first time in 2005 from rhizosphere soil of spring barley (*Hordeum vulgare* L.) (Johansen et al. 2005), has been described as a soil bacteria (Leigh et al. 2006; Wang et al. 2009) that degrades cephalomannine (Li et al. 2007) and xenobiotic pollutants such as polychlorinated biphenyl (PCB) (Leigh et al. 2006). Herein, we describe, for the first time, the ability of this new genus to produce lipase with broad biotechnological potential. In our study, lipase production by *Luteibacter* sp. was improved with culture on low-cost media. We also characterized the lipolytic activity profile produced in the culture supernatant to attest to the potential of the lipase for biotechnological purposes.

Materials and methods

Microorganisms

Luteibacter sp. isolated from biofilms on PCB oils that originated from soil collected in a tar spot in Germany, (52°N39.923', 09°E 48.563') (Macedo et al. 2007) was used in this study. The strain was maintained on agar slants in Luria– Bertani medium [LB (g/L): tryptone, 10; yeast extract, 5;, sodium chloride, 10]. The 16S rRNA gene sequence was amplified by PCR and sequenced for molecular identification as described previously (Abraham et al. 1999). The reactions were evaluated on a model 377 genetic analyzer (Applied Biosystems, Foster City, CA), and the software program SEQUENCHERTM ver. 4.0.5 (Gene Codes Corp., Ann Arbor, MI) was used to analyze the sequences. The sequences were matched in BLAST 2.2.9 (Altschul et al. 1990) against the EMBL database (Kanz et al. 2005). The sequence was deposited at GenBank (NCBI) under accession number FR714940. Lipase production and culture conditions

The basal medium for lipase production consisted of 10 % LB (LB medium diluted in distilled water). All lipid sources used in this study were previously sterilized by dry heat (180 °C, 60 min) and added to the basal medium after the latter has been sterilized.

Growth experiments were performed in triplicate in 125mL Erlenmeyer flasks containing 30 mL of medium for 72 h in an orbital shaker set at 150 rpm and 30 °C without surfactant. The culture medium containing 2 % (v/v) of different vegetable oils (babasu, canola, corn, cotton, linseed, olive, rice, or soybean) or animal fats (pig or bovine) was inoculated with 100 μ L (OD₆₀₀=0.3) of *Luteibacter* sp. pre-inoculum (grown overnight in 10 % LB). The composition and contents of saturated and unsaturated fatty acids differ in these vegetable oils and animal fats. For example, linseed oil contains >50 % linolenic acid (omega 3), and soybean and canola oil contains between 8–10 % linolenic acid (omega 3). Bovine fat contains 20–37 and 25–40 % of the satuated fatty acids palmitic acid and stearic acid, respectively (Zambiazi et al. 2007).

Based on the results of the growth experiments, we chose the two best lipid sources for lipase production—olive oil and bovine fat—and tested these at different concentrations [1, 2, 3, and 4 % (v/v)] in culture media. Five different pH values were tested (pH 5, 6, 7, 8, 9) to determine the best initial pH for lipase production. The initial pH of the medium was adjusted with HCl or NaOH 1 M. Different growth temperatures (25, 30, and 37 °C) for lipase production were also tested.

The effect of different surfactants [Triton X-100, Tween 80, Tween 20, sodium dodecyl sulfate (SDS); 0.25 % (v/v) (Silva et al. 2005); all from Sigma Chemical Co., St. Louis, MO] on lipase extraction was evaluated. The respective surfactant was added to the medium after bacteria growth, shaken vigorously (Silva et al. 2005), and then centrifuged (14,000 rpm, 7 min). The lipase-rich extract obtained without surfactant addition was extracted and denoted "lipases in culture supernatant" (LCS). Lipase-rich extract obtained with the addition of, for example, Tween 80 at a concentration of 0.25 % was named "lipase in culture supernatant with Tween 80" (LCST80).

Analytical assays

Lipase activity in LCS and LCST80 was assayed using ρ nitrophenyl palmitate (ρ NPP; Sigma Chemical. Co.). Samples (LCS or LCST80, 20 μ L) were mixed with 80 μ L of substrate solution at pH 8.0 (0.3 mg/ml ρ NPP) (Silva et al. 2005). After 30 min incubation at 37 °C, the absorbance was measured spectrophotometrically at 410 nm using a Spectramax plate spectrophotometer (Molecular Devices, Sunnyvale, CA) and an enzyme-free control. Enzyme activity was determined with reference to a calibration curve of standard ρ -nitrophenol (Sigma Chemical Co.). One lipase unit (U) was defined as the amount of enzyme that releases 1.0 μ mol ρ -nitrophenol per liter per hour. All enzyme assays were carried out in triplicate, and the average values were calculated.

LCS and LCST80 characterization

The enzyme characterization assays were performed using pNPP as the substrate, as described above. The optimal temperature for enzymatic activity of LCS and LCST80 was tested at different temperatures (25, 30, 37, 45, and 50 °C). The pH stability of lipolytic activity was determined by pre-incubating the LCS and LCST80 solutions at different pH values (3-10) at 45 °C for 30 min before the lipase activity assay. The buffers used were citric acid/sodium phosphate (pH 3-7), Tris-HCl (pH 7-9), and sodium carbonate/sodium bicarbonate (pH 10.0), all at a final concentration of 25 mM. The effect of metal ions was determined by pre-incubating the LCS and LCST80 solutions at 45 °C for 30 min before the lipase activity assay with different chloride salts, namely, cobalt, magnesium, zinc, and calcium, at a concentration of 5 mM. Triton X-100 (Sigma Chemical Co.), and SDS, both at 0.5 %, were used to study the effects of detergents on the activity of LCS and LCST80 by pre-incubating the LCS and LCST80 solutions with each of these detergents at 45 °C for 30 min before the lipase activity assay. The effect of other chemical agents, such as methanol (Merck, Darmstadt, Germany) and the chelating agents EDTA and EGTA (0.5 %, v.v) (all from Sigma Chemical Co.) on LCS and LCST80 activity was determined under the same conditions described above.

For our evaluation of enzyme substrate specificity, we different ρ -nitrophenyl ester substrates, including ρ NP-stearate (ρ NPS), ρ NPP, ρ NP-myristate (ρ NPM), ρ NP-butyrate (ρ NPB), and ρ NP-acetate (ρ NPA) (all from Sigma Chemical Co.) as described above.

Transesterification activity was assayed as described previously by Teng and Xu (2007). For both, 1mL samples of LCS and LCST80 were lyophilized until completely dry at -50 °C, 0.04 mbar in an ALPHA 1–4 LD plus freeze dryer (Martin Christ GmbH, Osterode am Harz, Germany), and the dry supernatant was used in the experiments. Enzyme activity was determined with reference to a calibration curve of standard ρ -nitrophenol (Sigma Chemical Co.). One lipase unit (U) was defined as the amount of enzyme that releases 1.0 µmol ρ -nitrophenol per liter per hour. All enzyme assays were carried out in triplicate and the average values were calculated.

The lipase assay using the titration of fatty acids was performed according to Colen et al. (2006). One lipase unit (U) was defined as the amount of enzyme that releases $1.0 \mu mol$ fatty acid under assay conditions. Controls were

performed with distilled water instead of LCS and LCST80. All assays were carried out in triplicate, and the average values were calculated.

Statistical analysis

Statistical significance of the treatments was determined using one-way analysis of variance (ANOVA), and mean separations were compared using Tukey's test (P < 0.05) with statistical analysis software (Graphpad Prism 3.0 Software Inc., San Diego, CA, USA).

Results and discussion

Molecular identification, production, and lipase extraction

The molecular analysis of the 16S rDNA gene revealed high similarity among bacteria belonging to genus Luteibacter sp., with 99 % similarity with LJ96 Luteibacter rhizovicinus (data not shown). Several studies have shown an increase in lipase production when oils are used as inducers of enzyme secretion (Ciudad et al. 2011). In our study, variations in carbon substrates, surfactant, pH, and temperature had significant effect on the level of lipase activity. Among the lipid sources tested here, olive oil showed the best results in terms of lipase production (Fig. 1a). Wang et al. (2008) demonstrated that the lipase production-inducing capability of olive oil can be attributed to its composition - specifically to its high content (>70 %) of oleic acid. Olive oil has also been described as a good lipid source for lipase production by other microorganism (Ota et al. 1982; Wang et al. 2007; Mafakher et al. 2010; Yadav et al. 2011), including a thermophilic lipase from Burkholderia cepacia (Lu et al. 2009).

Since Brazil has the largest commercial cattle herd in the world, bovine fat is an abundant and also commercially strategic alternative substrate for lipase production. We therefore tested the effect of different concentrations of olive oil (the best lipid source) and bovine fat (an alternative substrate). The amount of olive oil influenced lipase secretion: the addition of 2, 3, and 4 % of olive oil to the basal medium resulted in higher lipase activity $(50.31\pm3.45,$ 45.46 ± 5.53 , and 50.00 ± 1.75 U/L h, respectively); in contrast, lipase activity was inversely proportional to the amount of bovine fat added to the medium, with lipase activity reaching 43.94±1.75 U/L h with 1 % bovine fat (Fig. 1b) and decreasing in a linear manner in the presence of higher concentrations of this substrate. Other substrates have been successfully used for lipase production, including tallow (Papanikolaou et al. 2007; Ramani et al. 2010), rapeseed oil (Kamzolova et al. 2005), and waste cooking oil (Papanikolaou et al. 2011).

Fig. 1 Effect of lipid sources on Luteibacter sp. lipase production in basal medium at 30 °C and 150 rpm for 72 h. a Lipase production using different lipid sources at a concentration of 2 %, **b** lipase production using olive oil and bovine fat at different concentrations. All experiments were performed in triplicate. Results are presented as the mean with standard deviation (SD; error bars). Means denoted by the same uppercase letter are not significantly different according to Tukey's test ($\alpha = 0.05$)



Olive oil at a concentration of 2 % enhanced lipase production at varying pH and different temperatures. The optimal initial pH was 6, while at pH 5, 7, 8, and 9 the lipase activity was approximately half of the value obtained at pH 6 (Table 1). This result is in accordance with other lipase-producing bacteria, such as *Bacillus* sp. (Ertugrul et al. 2007) and *Geobacillus* sp. (Ebrahimpour et al. 2008). The culture temperature also strongly influenced lipase production, since lipase activity increased threefold at 37 °C when compared to 25 °C (Table 1), as also reported for other bacteria (Gupta et al. 2004).

It is well documented that the addition of surfactants can increase cell permeability and facilitate contact between enzyme and substrate, probably changing the conformation of the lipase or interfacial properties, thereby increasing lipase activity (Helisto and Korpela 1998; Sharma et al. 2001; Gupta et al. 2004; Silva et al. 2009). The addition of surfactants for lipase extraction also increases the concentration of enzymes in the culture supernatant, allowing the release of enzymes that are associated with the external cell surface, as described for conidial surface lipase from

 Table 1 Effect of pH and temperature on lipase production by

 Luteibacter sp.

ulture condition Lipase activity (
pH ^a	
5.0	49.50±1.7 5a
6.0	90.92±3.83 b
7.0	49.63±3.95 a
8.0	54.75±2.45 a
9.0	39.40±3.03 c
Temperature (°C) ^b	
25	44.45±3.50 a
30	94.71±4.79 b
37	146.23±6.72 c

Data are presented as the mean \pm standard deviation (SD). All experiments were performed in triplicate. Means followed by the same letter are not significantly different according to Tukey's test (α =0.05)

 $^{\rm a}$ Basal medium plus 2 % olive oil, incubated at 30 $^{\circ}{\rm C}$ in shaker at 150 rpm, for 72 h

^b Basal medium plus 2 % olive oil, adjusted to pH 6, incubated in shaker at 150 rpm, for 72 h

Metarhizium anisopliae (Silva et al. 2009; Santi et al. 2010). The addition of 0.25 % Tween 80 to the culture (produced at pH 6, with 2 % olive oil, at 37 °C) before centrifugation resulted in an 80 % increase in lipase activity, while the addition of 0.25 % SDS reduced lipase activity by approximately 30 % of the original activity. Similar to *Serratia marcescens* (Zhao et al. 2008), in our study lipase activity in *Luteibacter* sp.was strongly inhibited by Triton X-100.

Comparing the values of lipase activity during the initial production experiments with those obtained after improvement to the lipase production and extraction conditions, we found that lipase activity had increased by about 215 U/L h or 427 % under the improved conditions. These results attest to the relevance of culture conditions and extraction procedures for enzyme production. Lipase production may be increased still further by optimization studies related to other culture parameters, such as aeration or production in a bioreactor where factors related to bioreactor design can also be modified to increase enzyme production.

Characterization of LCS and LCST80 activity

Given the improvement in lipase activity caused by the addition of Tween 80 to the culture for lipase extraction, we decided to characterize the activity of this culture supernatant, which we named LCST80. As expected, the enzymes present in the culture supernatant acted differently on synthetic substrates of different acyl chain lengths (Fig. 2). Both the LCS and LCST80 extracts hydrolyzed all tested substrates, with the same pattern of activity; however, the lipase activity in LCST80 was always approximately 1.4- to 2.7-fold higher than that of LCS using the same substrates. LCS and LCST80 showed the highest hydrolysis on ρ NPA (2 carbons in the fatty acid chain), as previously described for *Metarhizium*



Fig. 2 Effect of acyl length on LCS and LCST80 lipase activity. ρ -Nitrophenyl ester substrates used: ρ NP-acetate (ρ NPA; 2C), ρ NPbutyrate (ρ NPB; 4C), ρ NP-myristate (ρ NPM; 14C), ρ -nitrophenyl palmitate (ρ NPP; 16C), ρ NP-stearate (ρ NPS, 18C). Results are presented as the mean of three activity determinations. *Error bars* SD. *LCS* Lipases in culture supernatant (lipase-rich extract obtained without surfactant addition), *LCST80* lipases in culture supernatant with Tween 80 (lipase-rich extract obtained with the addition of Tween 80 at a concentration of 0.25 %)



Fig. 3 Effect of temperature on LCS and LCST80 lipase activity. The hydrolytic activity on ρ NPP was determined at the indicated temperatures in 50 mM Tris–HCl buffer pH 8.0 and is expressed relative to the maximal activity. Results are presented as the mean of three activity determinations. *Error bars* SD

anisopliae spore surface lipase (MASSL) (Silva et al. 2009). Lipases can act on substrates with short and long fatty acid chains, but the capability to hydrolyze substrates with long acyl chains is a fundamental difference between lipases and esterases (Fojan et al. 2000; Silva et al. 2009). The high activity on short fatty acid chains points to a possible presence of esterases or more than one lipase.

The presence of lipase activity on all tested substrates is an interesting characteristic for biotechnological applications of *Luteibacter* sp., such as detergent formulations and waste

Table 2 Effect of ions, detergents or organic solvent on LCS and LCST80 lipase activity $^{\rm a}$

Cation, detergent or organic solvent	Residual lipase activity (%)				
	LCST80	LCS			
Control	100.00 ± 7.44	100.00 ± 0.00			
Ca ²⁺ 5 mM	110.53 ± 10.53	198.20±12.21			
$Zn^{2+} 5 mM$	315.79±7.44	505.15±10.33			
Co ²⁺ 5 mM	60.53±18.61	$78.30{\pm}2.08$			
Mg ²⁺ 5 mM	113.16±3.72	135.71 ± 10.10			
EDTA 5 mM	110.53±7.44	155.40 ± 4.30			
EGTA 5 mM	163.16±9.12	170.33±5.25			
Triton X-100 0.5 %	114.03 ± 3.04	228.57±57.14			
SDS 0.5 %	282.46±12.15	521.43±10.10			
Methanol 0.5 %	165.79±3.72	264.29±30.30			

Data are presented as the mean \pm SD (based on three replicates)

LCS, Lipases in culture supernatant (lipase-rich extract obtained without surfactant addition); LCST80, lipase in culture supernatant with Tween 80 (lipase-rich extract obtained with the addition of Tween 80 at a concentration of 0.25 %); EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; SDS, sodium dodecyl sulfate,

^a LCS and LCST80 were pre-incubated at 45 °C for 30 min with different ions, detergents, or organic solvents. Activities are expressed as residual activity relative to control made with LCS or LCST80 plus distilled water instead of chemical compounds



Fig. 4 Effect of pH on LCS and LCST80 lipase stability. Both extracts were pre-incubated at 45 °C for 30 min in various buffers at different pH. Activities are expressed as residual activity relative to the control made with LCS or LSCT80 using distilled water instead of buffer. Results are presented as the mean of three activity determinations. *Error bars* SD

treatment, which require the ability to hydrolyze substrates of different lipid compositions and diverse acyl chain lengths (Hasan et al. 2006).

LCS and LCST80 showed elevated activity with increased temperature, with, 45 °C being the optimal temperature for lipase activity (Fig. 3). The optimum temperature of enzyme activity is an important factor, since several industrial processes require specific temperatures, or even heating, during one or more steps. In the same way, the influence of additives on lipase activity is very important, since the enzymes are used, in most cases, in different liquid formulations, as seen in enzymatic detergents (Hemachander and Puvanakrishnan 2000; Rathi et al. 2001). In this context, we tested the effect of ions, detergents, and organic solvents on LCS and LCST80 activity. Both extracts showed an increase in lipase activity in the presence of divalent cations, with the exception of Co^{2+} which decreased lipase activity by approximately 22 and 39 % in LCS and LCST80, respectively (Table 2). The presence of metal ions, especially calcium, has been reported to function

Ann Microbiol (2014) 64:251-258

as enhancers of enzyme production (Sethi et al. 2013). Calcium often stimulates lipase activity, and suggested pathways are via the removal of the hydrolysis product by the formation of an insoluble complex of calcium salts and fatty acids or alternatively simply by enzyme stabilization (Gupta et al. 2004; Silva et al. 2009). The chelating agents EDTA and EGTA did not inhibit lipase activity, suggesting that lipase activity produced is not metal dependent (Table 2). Likewise, the presence of an organic solvent (methanol) and detergents (SDS and Triton) did not affect lipase activity in LCS and LCST80 at the concentrations tested (Table 2). These results support the potential use of this lipase in chemical synthesis processes.

In the stability assay of lipase activity at different pH values, both LCS and LCST80 extracts showed the highest lipase activity at pH 7, but that in LCS was approximately 26 % higher than that in LCST80 (Fig. 4). LCS was stable at both acidic and alkaline pH, but the lipase activity in LCST80 fell by approximately 50 % at acidic pH (3–5); at pH 6 there was an increase in lipase activity that was sustained at alkaline pH (Fig. 4). Compared to *Y. lipolytica*, a yeast which has been well studied for lipase production (Pereira-Meirelles et al. 1997), lipases from *Luteibacter* sp. had a better stability at higher pH.

Lipase activity using natural lipids as substrates and transesterification assay

In the titration assay, LCS and LCST80 showed lipase activity on different natural oils and bovine fat, and the best results were obtained at 30 °C (Table 3). The results obtained in the titration assay indicate the presence of lipase activity in LCS and LCST80 for different natural lipids, demonstrating the biotechnological potential for biodiesel production. Furthermore, the activity on substrates with varied lipid composition is an important characteristic for

Natural substrates	Lipase activity (U/L h)						
	30 °C		37 °C		45 °C		
Substrate	LCST80	LCS	LCST80	LSC	LCST80	LCS	
Canola	$10.0 {\pm} 0.0$	15.0 ± 7.1	$10.0 {\pm} 0.0$	$10.0 {\pm} 0.0$	$10.0 {\pm} 0.0$	16.7±5.8	
Corn	14.0 ± 5.5	33.3±5.8	$10.0 {\pm} 0.0$	15.0 ± 7.1	16.7 ± 5.8	20.0 ± 0.0	
Olive	16.7±5.8	35.0±7.1	23.3±5.8	23.3 ± 5.8	20.0 ± 10.0	25.0±7.1	
Soybean	$10.0 {\pm} 0.0$	$20.0 {\pm} 0.0$	$10.0 {\pm} 0.0$	$20.0 {\pm} 0.0$	16.7 ± 5.8	16.7±5.8	
Bovine fat	26.7±5.8	$50.0 {\pm} 0.0$	$0.0 {\pm} 0.0$	$0.0 {\pm} 0.0$	$10.0 {\pm} 0.0$	$0.0 {\pm} 0.0$	
Mixture of oils	13.3 ± 5.8	$150.0 {\pm} 0.0$	15.0 ± 7.1	$0.0{\pm}0.0$	$0.0{\pm}0.0$	10.0±0.0	

Table 3 LCS and LCST80 lipase activity on different natural substrates

Data are presented as the mean \pm SD. All experiments were performed in triplicate

Titration of fatty acids using vegetable oils and bovine fat incubated at different temperatures, for 30 min at 150 rpm in the presence of LCS or LCST80

detergent lipases, which have to act on mixtures of different lipids (Rathi et al. 2001).

Vegetable oils and animal fats have a relatively high viscosity, which is a disadvantage for their use in several commercial applications, such as for alternative fuels. For this reason, transesterification is the central reaction of biodiesel production because the replacement of glycerol with an alcohol molecule reduces the viscosity of the oil (Meher et al. 2006; Ranganathan et al. 2008; Bajaj et al. 2010). Both LCS and LCST80 showed transesterification activity on ρNPP (16C) and ρNPM (14C). The lipase activity of LCS was 2.270±0.110 and 3.124±0.459 U/L h on pNPP and ρ NPM, respectively, whereas that of LCST80 was 2.078± 0.714 and 2.511±0.306 U/L on pNPP and pNPM, respectively. The use of enzymes for transesterification enables the synthesis of specific alkyl esters, easy recovery of the glycerol, and reaction with triglycerides with high free fatty acid content (Bajaj et al. 2010).

Increased knowledge on lipase production, isolation of new lipase-secreting bacteria, and characterization of their lipolytic activity are very important steps to consolidate these enzymes in different commercial sectors. The characterization of lipase activity produced by *Luteibacter* sp. introduces this recently described genus as a new source of lipases with great biotechnological potential. Future studies on the isolation and characterization of *Luteibacter* sp. lipases could facilitate the application of these enzymes in other industrial applications. The data presented here will be used for further improvement of lipase production in a pilot plant bioreactor using different operational strategies.

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