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ORIGINAL PAPER

A novel thermostable lipase from Basidiomycete *Bjerkandera adusta* R59: characterisation and esterification studies

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Abstract Microbial lipases are widely diversified in their enzymatic properties and substrate specificities, which make them very attractive for industrial application. Partially purified lipase from *Bjerkandera adusta* R59 was immobilized on controlled porous glass (CPG) and its properties were compared with those of the free enzyme. The free and immobilized lipases showed optimal activities at 45 and 50°C, respectively. Both enzyme forms were highly thermostable up to 60°C. The enzymes were stable at pH from 6.0 to 9.0 and their optimal pH for activity was 7.0. The free lipase was more thermostable in *n*-hexane than in aqueous environment. Both lipase preparations had good stabilities in non-polar solvents and were capable of hydrolysing a variety of synthetic and natural fats. Non-immobilized lipase activity was inhibited by disulphide bond reagents, serine and thiol inhibitors, while EDTA and eserine had no effect on enzyme activity. All anionic detergents tested in experiments inhibited lipase activity. The free lipase showed good stability in the presence of commercial detergents at laundry pH and temperatures. Applications of free and immobilized lipases for esterification were also presented.

Keywords Immobilized lipase · *Bjerkandera adusta* R59 · Ester synthesis · Enzymatic properties

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Introduction

The interest in lipases arises due to their ability to catalyze the hydrolysis of triacylglycerols and the synthesis of esters from glycerol and long-chain fatty acids. The enzymes exhibit the phenomenon of interfacial activation—they are active at the interface between their hydrophobic lipid substrate and the hydrophilic medium (oil–water interface) [40].

These enzymes have been found in many species of animals, plants and microorganisms. Among microorganisms, fungi are important lipase producers since they generally produce extracellular enzymes, which facilitates the enzyme recovery from the medium [10, 13, 22, 41].

Lipases are used extensively in biotechnological fields such as food technology, clinical and industrial chemistry [16, 31]. New applications are being developed based on the ability of lipases to catalyse synthesis reactions in systems with very low water concentrations [1, 12, 29].

The aim of the present work was a comparative study of biocatalytic properties of partially purified free and immobilized lipases from *Bjerkandera adusta* R59. We evaluated the stability of both lipase forms in organic solvents and we investigated the effect of various reaction parameters on the lipase-catalysed esterification reactions between aliphatic alcohols and fatty acids in *n*-hexane.

Materials and methods

Chemicals

Analytical reagent grade chemicals were obtained from Sigma (St Louis, MO, USA), Merck (Darmstadt, Germany), Fluka

(Buchs, Switzerland), POCh (Gliwice, Poland), Pharmacia (Uppsala, Sweden), Riedel-de Haën (Darmstadt, Germany).

Oils were purchased from commercial sources.

Microorganism

Lipase-producing microorganism was previously isolated from a soil sample from a field near Lublin (south-eastern Poland) [11], identified as *B. adusta* R59 and deposited in the Culture Collection of Basidiomycetes (Praque, Czech Republic) under accession number CCBAS930 and nucleotide sequences are available in GenBank under accession number AY 319191.

Medium and growth conditions for lipase production

Recently, we have optimized the production of lipase from a newly isolated strain *B. adusta* R59 [11]. Growth medium contained 0.5% sucrose instead of glucose in a basal GKM medium (glucose–KH_2PO_4–$MgSO_4$ medium). The culture medium was autoclaved at 121°C (15 psi) for 25 min. After autoclaving, the pH of the medium was adjusted to 6.0 by addition of sterile KOH solution. The culture was grown at 30°C for 48 h on a rotary shaker.

Enzyme preparation

At the end of incubation, the cells were removed from the culture medium by centrifugation (10,000 rpm for 20 min). The cell-free supernatant was partially purified by gel filtration on Sephadex G-25 column (7×40 cm) which was previously equilibrated with 0.1 M buffer Tris–HCl pH 7.0. Lipase-containing fractions were pooled and analysed for enzyme activity. The active fractions were freeze-dried and used for later studies and ester synthesis.

Lipase immobilization

One gram of silanized controlled porous glass (CPG) was activated by glutaraldehyde (5 ml) at concentration of 5% with 150 mg of $NaBH_4$. This reaction mixture was shaken gently at room temperature for 30 min, then the beads were washed three times with 0.01 M phosphate buffer pH 7.0. Such obtained CPG beads and 30 mg of lyophilized enzyme (containing 10 mg of protein and specific lipase activity was 106.6 U/mg protein) were mixed and incubated for 3 h at room temperature. Finally, the immobilized enzyme was washed five times with 500 ml of 0.01 M phosphate buffer pH 7.0. The immobilized lipase was then stored at 4°C for further use.

Measurement of lipase activity

The activity was determined according to the method of Sokolovska et al. [34] with slight modification [11]. One unit of enzyme activity was defined as the amount of the lipase that produced 1 μM of free fatty acids per minute at 45°C and pH 7.0.

Physicochemical properties of free and immobilized lipase

To determine the optimal temperature for enzymic activity, free and immobilized enzymes were incubated at different temperatures (20–90°C) at pH 7.0. The thermostability of lipases were determined by pre-incubating the enzymes at temperatures ranging from 30 to 90°C for 120 min.

The pH optimum of the free and immobilized lipase was measured by using the 50 mM Britton–Robinson buffer [8]. To analyse the pH-stability of the lipase, free and immobilized enzyme were stored at room temperature for 24 h in 50 mM Britton–Robinson buffer at pH 3.0–11.0. The residual activity was determined at pH 7.0.

The thermostability of native lipase in organic solvent was determined by measuring the enzyme activity in *n*-hexane at different temperatures (60–90°C).

The substrate specificity of the both enzyme forms towards various triacylglycerols and oils was analysed by titrimetric assay in phosphate buffer pH 7.0.

Positional specificity was analysed by TLC of the enzymatic reaction products obtained with pure triolein (99% purity). About 200 μg of lipase, 20 mg of pure triolein and 2 ml of 50 mM phosphate buffer pH 7.0 were mixed and incubated for 120 min at 60°C with shaking. After incubation, 1.5 ml of ethyl ether was added to the reaction mixture to extract the reaction products. Aliquots of the ether extract were applied to TLC plates (silica gel-60; Merck, Darmstadt, Germany). The liquid phase consisted of chloroform/acetone/acetic acid (96:4:1). The spots were visualized by iodine vapours and detected with a videocamera (Camag).

Effect of various compounds on enzyme activity and stability

Enzyme assays to test the effect of inhibitors on the native lipase activity were performed in the presence of various agents at concentrations of 1 and 10 mM. The reaction mixtures were incubated for 30 min at room temperature and the residual activity was measured quantitatively with respect to a control.

To analyse the lipase stability in the presence of organic solvents, both free and immobilized enzymes were incubated in 50 mM phosphate buffer (pH 7.0) in the presence of various solvents (at a final concentration of 50%). After 24 h incubation at room temperature residual activity was determining using titrimetric method.

To investigate the effect of surfactants on the lipase activity, the enzyme samples were incubated in the presence of

specific detergents at concentrations of 1 and 10 mM for 30 min at room temperature. The effect of hydrogen peroxide at concentrations up to 1% v/v on the enzyme activity was also investigated.

Lipase stability was checked in the presence of commercial detergents (Ariel, Vizir, Jelp) at a concentration of 10 mg/3 ml for 60 and 90 min at 50 and 60°C and pH 10.0 and 11.0. Calcium chloride was added to the assay mixture at a concentration of 1 mM as stabilizer. The residual lipase activity was determined under standard assay conditions.

The activity assayed in the absence of chemical agents was defined as a control.

Synthesis of esters by *Bjerkandera adusta* R59 lipase

For a comparative study, free and immobilized lipases were used as biocatalysts for the esterification. The reaction mixture was composed of 50 mM fatty acids (oleic, caprylic), 150 mM aliphatic butanol and 10 ml of *n*-heptane. About 360 U of free lipase or 70 U of immobilized enzyme were added to the reaction mixture. The reactants were incubated at 45 (with native enzyme) or 50°C (with immobilized biocatalyst) in a shaker at 150 rpm for 72 h. The residual acids contents were assayed by titration with 0.05 N KOH using phenolphthalein as an indicator. Control experiments were conducted without the addition of the biocatalyst. The conversion (%) of ester synthesis was calculated based on the amount of acid consumed from the reaction medium.

Results and discussion

Immobilization of lipase on CPG

The result of covalent immobilization of *B. adusta* R59 lipase on activated controlled porous glass is shown in Table 1. In total 4.7 mg lipase was covalently attached per g of CPG under the above immobilization conditions and 53% protein was recovered in subsequent washings as unbonded enzyme. The immobilized lipase showed good hydrolysis activity of 250 U/g of CPG.

Table 1 Activity of immobilized lipase from *Bjerkandera adusta* R59

Properties	Native	CPG-immobilized
Yield (%)[a]	100	47
Hydrolysis activity (%)[b]	100	49.8

[a] Yield is defined as the percentage of enzyme attached to CPG relative to that added initially to the reaction

[b] Hydrolysis is determined towards tributyrin, as described in "Materials and methods"

Effect of temperature on activity and stability of the free and immobilized lipase

The lipase from *B. adusta* R59 was active in the temperature range 20–80°C with maximal activity at 45°C for free enzyme (data not shown). The optimum temperature for immobilized lipase was 50°C.

The thermal stabilities of soluble and immobilized lipases were determined after incubation for 120 min at different temperatures with tributyrin as substrate. Both native and immobilized lipase from *B. adusta* R59 was highly thermostable up to 60°C (Fig. 1a). The free lipase retained more than 40% of the maximum activity at 70°C, while the immobilized enzyme retained 57% activity under these conditions. Similarly, native lipases from *Penicillium expansum* [36] and *Antrodia cinnamomea* [33] had temperature optima at 45°C. The thermostability was also similar to the report on free lipase from bacteria *Bacillus thermoleovorans* ID-1 [19], which was stable up to 50°C. Good thermostability have been reported for lipases from *Aspergillus terreus* [42] and *Penicillium wortmanii* [7]. Activity and thermostability of the *B. adusta* R59 immobilized lipase were similar to the properties of other immobilized lipases from *Bacillus* sp. [9], *Rhizopus* sp. [35] and *Candida rugosa* [24]. The results obtained in our work indicated that the *B. adusta* R59 lipase was stable at high temperatures. Moreover, it has been demonstrated that the thermostability of lipase from *B. adusta* R59 was enhanced by immobilization.

Effect of pH on activity and stability of the free and immobilized lipase

Lipolytic activity of free and immobilized enzymes were maximal at pH 7.0 and high over the range of pH 4.0–10.0 (enzymes exhibited over 50% of their maximal activity) (data not shown). The pH stability of enzymes is shown in Fig. 1b. When the both enzyme forms were incubated at various pH values for 24 h, we observed that the lipases could tolerate a broad range of pH values from 3.0 to 11.0. Native lipase retained more than 30% activity between pH 6.0 and 9.0, while for the immobilized enzyme more than 30% residual activity was obtained in the range of pH 5.0–9.0 (Fig. 1b). Immobilized enzyme from *B. adusta* R59 was more pH-stable than free lipase. The results are in agreement with the previous ones for free and immobilized intracellular lipases from *Rhizomucor miehei* and *Yarrowia lipolytica* [3]. Stability between pH 4.0 and 9.0 has been also reported for native lipases from *Aspergillus terreus* [42], *Mucor hiemalis f. hiemalis* [15]. pH stability of immobilized lipase from *Bacillus* sp. [9] was shifted towards a higher range (pH range 8.0–10.0) in comparison with immobilized lipase from *Bjrkandera adusta* R59 strain.

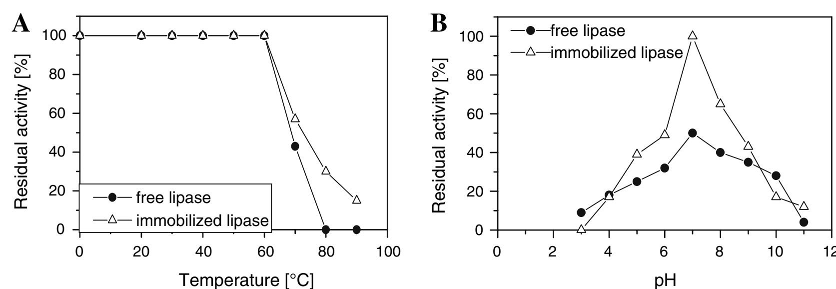


Fig. 1 The effect of temperature (**a**) and pH (**b**) on the stability of free and immobilized lipase. For determining thermostability the activity of both lipase forms was assayed after 120 min of incubation at different temperatures up to 90°C at pH 7.0. For pH stability residual activity was measured after 24 h of incubation at different pH with the standard titrimetric assay. Each point is the average of two duplicate tests and the maximum deviation in the data was no more than 10%

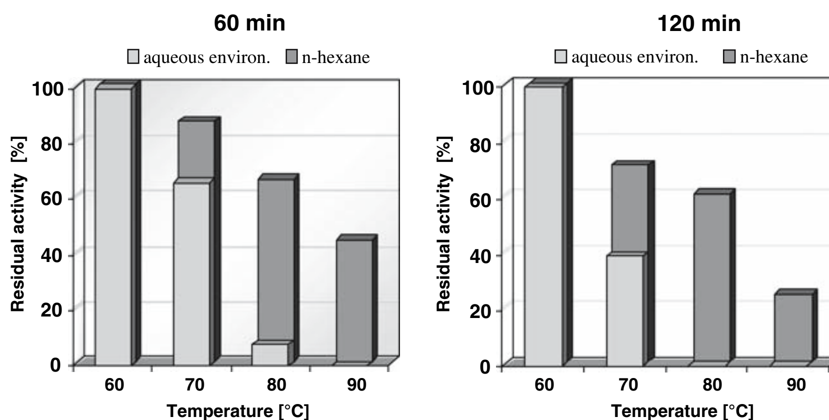
Thermostability in pure *n*-hexane

Thermostabilities of the native enzyme in aqueous environment and pure *n*-hexane are compared in Fig. 2. In organic solvent, we observed an enhancement of thermal stability of native lipase. The enzyme from *B. adusta* R59 was highly stable in pure *n*-hexane in comparison with the thermostability of the biocatalyst in aqueous solution. After 120 min of incubation at 80°C in *n*-hexane, the lipase retained 60% activity, whereas in aqueous environment the enzyme was totally deactivated under these conditions (Fig. 2). Similar results have been reported for *Burkholderia cepacia* lipase in organic solvent [27]. The enzyme was highly stable at 80°C in 1.5 ml *n*-heptane—after 1 h the remaining activity was 55% for free lipase.

Stability in organic solvents

The stability in organic environment is an important property of lipases. The effect of the organic medium depends on the nature of both enzyme and solvent [14]. The stability of native and immobilized lipase from *B. adusta* R59 was investigated in various polar and non-polar organic solvents (log *P* ranging from −1.30 to 4.0) at a final concentration of 50%. Figure 3 shows the residual activity of the both enzyme forms after exposure to organic solvents. We demonstrated that lipase activity was highly dependent on hydrophobicity of solvents. The aqueous and immobilized lipases had good stabilities in water-immiscible (non-polar) organic solvents such as cyclohexane, *n*-hexane and *n*-heptane. In *n*-hexane and *n*-heptane the enzymes were activated, with the residual activities greater than 100% (Fig. 3). Water-immiscible solvents ($2 < \log P < 4$) allow the enzymes to retain the catalytic activity due to the fact that they do not strip off the water layer from the surface of enzyme [14, 39]. In contrast, both native and immobilized biocatalysts from *B. adusta* R59 showed low stability in water-miscible (polar) organic solvents (Fig. 3). The same behaviour was found for the lipases from *Penicillium aurantiogriseum* [21], *Penicillium simplicissimum* [39], *Bacillus megaterium* [20] and for the immobilized lipases from *Mucor* sp. [2] and *Bacillus* sp. [26]. Interestingly, the lipases from *Bacillus* sp. [37] and *Pseudomonas cepacia* [38] presented an opposite behaviour: they were inhibited by *n*-hexane, water-immiscible solvent. In our experiments, the immobilized lipase from *B. adusta* R59 showed higher activity in organic solvents in comparison to that of the free enzyme.

Fig. 2 Comparative thermostability of free lipase from *B. adusta* R59 lipase in aqueous solution and organic solvent (*hexane*). The enzyme was incubated for 60 and 120 min at various temperatures. 100%—maximum activity of in aqueous environment assayed at 45°C. Data are mean from triplicate determinations



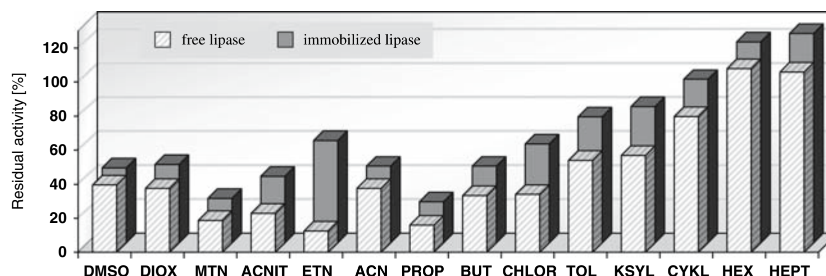


Fig. 3 The effect of organic solvents on the activity of the free and immobilized lipase. Data are mean from triplicate determinations. *DMSO* dimethyl sulfoxide (log *P* −1.30), *DIOX* dioxane (−1.10), *MTN* methanol (−0.76), *ACNIT* acetonitrile (−0.33), *ETN* ethanol (−0.24), *ACN* acetone (−0.23), *PROP* propanol (0.28), *BUT* butanol (0.80), *CHLOR* chloroform (2.0), *TOL* toluene (2.50), *KSYL* xylene (3.10), *CYKL* cyclohexane (3.20), *HEKS* hexane (3.50), *HEPT* heptane (4.0)

Substrate and positional specificities of the free and immobilized lipase

One of the most important properties of lipases is the substrate specificity towards triacylglycerols [28]. The hydrolytic rate of the free and immobilized lipases from *B. adusta* R59 towards synthetic triglycerides and vegetable oils was investigated. The both lipase forms hydrolysed all substrates tested with the highest activity on tributyrin (Table 2). These lipases from *B. adusta* R59 are highly specific for short chain synthetic triglycerides and hydrolysed them in order $C_4 > C_8 > C_{18:1}$. This was similar to the report on the enzymes from other strains such as *Candida rugosa* [5] and *Cryptococcus* sp. S-2 [17]. In contrast, the native lipase from *Mucor hiemalis f. hiemalis* [15] showed maximum activity towards rape oil. A high activity was also observed on tricaprylin.

Figure 4 shows a thin-layer chromatogram of the reaction products obtained from the triolein hydrolysis by the native lipase from *B. adusta* R59. The enzyme formed 1,2-diolein, oleic acid and traces of 1-monoolein. The absence of 1,3-diolein suggests that the lipase is specific for the primary position of the glyceride and showed 1,3-regiospecific nature. This is in accordance with studies on lipases from *Rhodotorula pilimanae* [25], *Aspergillus carneus* [30] and *Pseudomonas fluorescens* strain 2D [23]. Yadav et al. [42] also reported that lipase from *Aspergillus terreus* exhibited a strong specificity towards the outer chains of triolein. On the contrary, Sztajer et al. [39] showed that the lipase produced by *Penicillium simplicissimum* is non-specific and hydrolysed each of the three bonds of the triacylglycerol.

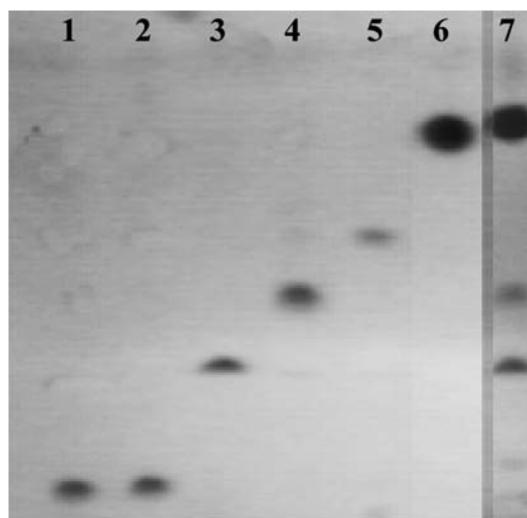


Fig. 4 TLC analysis of hydrolysis products after incubation of free *B. adusta* R59 lipase on triolein as substrate at 60°C for 120 min. *1* 1-Monoolein, *2* 2-monoolein, *3* oleic acid, *4* 1,2-diolein, *5* 1,3-diolein, *6* triolein, *7* hydrolysis products of triolein with lipase from *Bjerkandera adusta* R59

Table 2 Activity of the free and immobilized lipases from *B. adusta* R59 towards various substrates

Substrates	Remaining activity (%)	
	Free lipase	Immobilized lipase
Tributyrin (C4)	100[a]	100[b]
Tricaprylin (C8)	76	92
Triolein (C18:1)	42	78
Sunflower oil	42	46
Soybean oil	46	50
Olive oil	40	64
Rape oil	38	44
Corn oil	52	80

Lipase activities are expressed as the percentage of that of tributyrin, which was taken as 100%

[a] Initial activity of free lipase = 123 U/ml

[b] Initial activity of immobilized lipase = 250 U/g of CPG

In Table 3 the comparative physico-chemical properties of free and immobilized lipases from *B. adusta* R59 are presented. The both lipase forms (native and immobilized) had good activity and stability at high temperatures in aqueous environment. These enzymes were active in the broad pH range and they showed high stability in organic solvents with log $P > 3.0$.

Table 3 Comparative study of the physico-chemical properties of free and immobilized lipases

Physico-chemical property	Free lipase	Immobilized lipase
Optimum temperature	45°C	50°C
Temperature stability in aqueous solution (2 h)	up to 60°C	up to 60°C
Optimum pH	7.0	7.0
pH stability range (24 h)	6.0–9.0	6.0–9.0
Thermostability in *n*-hexane	up to 70°C	–
Stability in organic solvents (24 h)		
In 50% of *n*-hexane	108%	118%
In 50% of *n*-heptane	106%	123%
Substrate specificity	Tributyrin	Tributyrin
Positional specificity	1,3-Regiospecific	–

Effect of inhibitors on the native lipase activity

Studies on the effect of different active site-inhibitors on the lipase activity has provided of information on the action mechanism and nature of these enzymes. The effect of enzyme inhibitors upon native lipase activity was shown in Table 4. The native lipase was strongly inhibited by PMSF and *p*-nitrophenyl phosphate suggesting that this lipase belongs to the class of serine hydrolases. The lipase from *Aspergillus terreus* [42] and *Aspergillus carneus* [30] belongs also to the same class of enzyme. *B. adusta* R59 lipase showed high sensitivity to the disulphide bond reagents and lost approximately 20–30% of its total activity in the presence of these inhibitors at all tested concentrations (Table 4). This confirmed the presence of sulphur-containing amino acids in the active site of the lipase. The activity of the native enzyme from *B. adusta* R59 was also inhibited by thiol specific reagents (NEM, PCMB and iodoacetamide) at a concentrations of 1 and 10 mM. This suggested that the lipase require -SH group for its activity. 1,10-phenanthroline had an inhibitory effect on the lipase activity suggesting that Zn^{+2} ions are required for its catalytic activity, which is in agreement to report for the lipase from *Pseudomonas fluorescens* strain 2D [23]. The metal-chelating agent EDTA and the esterase inhibitor, eserine, appeared to have no significant effect on *B. adusta* R59 lipase activity at the concentrations tested, indicating that the lipase is not dependent on a metal cofactor at its active site (Table 4). In contrast, lipases from *Flavobacterium odoratum* [18] and *Penicillium chrysogenum* 9′ [4] are metal-dependent enzymes.

Effect of surfactants on the lipase activity

The effect of ionic, non-ionic and zwitterionic detergents on the native lipase activity from *B. adusta* R59 at concentrations of 1 and 10 mM was showed in Table 5. All the anionic detergents with the exception of sodium deoxycholate at a concentration of 10 mM inhibited the enzyme from *B. adusta* R59. SDS was found to be a strong inhibitor causing almost full inhibition of lipase activity (Table 5). The zwitterionic detergents CHAPS and cationic CTAB caused moderate inhibition of enzyme activity. The *B. adusta* R59 lipase was stimulated by all the non-ionic detergents (Table 5). Similar results have been reported for *Aspergillus*

Table 4 Effect of various inhibitors on the activity of the partially purified lipase from *B. adusta* R59

Inhibitors		Residual activity (%) at a concentrations of		
		1 mM	5 mM	10 mM
Serine protease inhibitors	PMSF	36.4	27.3	16.6
	p-Nitrophenylphosphate	45.0	36.4	27.3
Disulphide bond reagents	Cysteine	45.0	39.0	23.0
	$K_3Fe(CN)_6$	41.8	37.0	25.0
	DTT	45.8	41.6	33.3
Thiol specific reagents	NEM	45.8	45.8	33.3
	Iodoacetamide	43.7	39.6	33.3
	PCMB	59.0	58.3	45.8
Metal-chelating agents	EDTA	66.6	71.0	75.0
	o-Phenanthroline	29.2	27.1	12.5
Esterase inhibitor	Eserine	82.0	88.2	80.0

Residual activity was determined with tributyrin and expressed as the percent of the control value (with no addition). Values represent the mean of three replicates

PMSF phenylmethylsulfonyl fluoride, *DTT* dithiothreitol, *NEM* *N*-ethylmaleimide, *PCMB* *p*-chloromercuribenzoate, *EDTA* ethylenediamine tetraacetic acid

Table 5 Effect of detergents on the native lipase from *B. adusta* R59

Detergents	Residual activity (%) at a concentrations of	
	1 mM	10 mM
Control	100	100
Triton X-100	142	130
Tween 20	110	100
SDS	25	0
Cholate	48	65
Sodium deoxycholate	65	100
CTAB	100	75
CHAPS	80	60

Control—without addition of detergents. Values represent the mean of three replicates

terreus [42]. In contrast, Triton X-100 inhibited activity of the lipase from *Mucor* sp. [2] and *Rhizopus* sp. [35].

Effect of commercial detergents on the lipase stability

Lipases used in detergents should be active in the presence of surfactants and need to be thermostable and stable under alkaline pH because the pH of laundry detergents is generally in the range of 9.0–12.0. These enzymes should also have varying thermostability at laundry temperatures of 50–60°C [6, 32].

The stability of lipase from *B. adusta* R59 in the presence of various commercial detergents was shown in Table 6. The residual activity was the highest after incubation for 60 and 90 min at pH 10.0 and 60°C in the presence of all tested commercial detergents. The lipase was found to be highly stable (it retained 81% activity) in the presence of Jelp after 60 min of incubation at 60°C and pH 11.0. Sharma et al. reported that the compatibility of lipase from

Table 6 The lipase stability in the presence of commercial detergents after incubation for 60 and 90 min at 50 and 60°C and pH 10.0 and 11.0

Incubation time	Commercial detergents	Residual activity (%)			
		Temperature 50°C		Temperature 60°C	
		pH 10.0	pH 11.0	pH 10.0	pH 11.0
Control		100.0	100.0	100.0	100.0
60 min	ARIEL	57.0	48.0	66.0	56.0
	PERSIL	60.0	48.5	68.0	62.5
	JELP	65.7	48.5	70.0	81.2
90 min	ARIEL	47.0	40.0	65.0	50.0
	PERSIL	53.0	42.0	70.0	62.5
	JELP	59.0	48.0	70.0	75.0

Control—sample without commercial detergents. Values represent the mean of three replicates

Bacillus sp. RSJ-1 in the presence of commercial detergents was good [32]. The lipase was stable for 30 min at 50 and 60°C giving 79 and 64% residual activity, respectively.

Stability of the native lipase from *B. adusta* R59 was also checked in the presence of hydrogen peroxide. The stability of enzymes in the presence of H_2O_2 is an important property for the use of these as an additive in laundry detergent formulation. In our work, the H_2O_2 at all tested concentrations enhanced lipase activity when it was preincubated at pH 10.0 and lost only 3% activity when it was preincubated in the presence of the bleach agent at concentration of 1% at pH 11.0.

Synthesis of esters by *Bjerkandera adusta* R59 lipase

The production of butyl esters of fatty acids was catalysed by free and immobilized lipase from *B. adusta* R59. The highest conversion rate after 72 h was observed for the synthesis of 1-butyl caprylate by both free and immobilized lipase (93 and 97%, respectively). Butyl oleate was produced at maximum conversion of 89% with 360 U of free lipase. The immobilized enzyme (70 U) also showed high ability for the synthesis of 1-butyl oleate with maximum yield of 96.5% after 72 h conversion. In general, ester formation was higher with immobilized lipase from *B. adusta* R59 in comparison with the free enzyme. Similar results were presented for the free and immobilized lipases from *Bacillus* sp. [9]. The highest conversions of methyl oleate were observed with the both enzyme forms (50–60%) after 16 h.

We presented the activity and stability of the partially purified lipase from *B. adusta* R59, in both aqueous and organic environments in the presence of various chemicals. The present lipase was stable at temperatures up to 60°C, which can be useful for industrial application since many processes are performed at temperatures around 50°C. Based on the stability in the presence of surfactants, commercial detergents and H_2O_2 it can be used as an additive for detergent formulation. We have also shown that free and immobilized lipases from *B. adusta* R59 can be used to catalyse the synthesis of various esters. It should be stressed on a fact that *B. adusta* R59 strain (Basidiomycetes) used in our research has not yet been described as an extracellular lipase producer. The obtained results allow for assumption that the enzyme may have an industrial application.

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