

# Heterologous Expression and Biochemical Characterization of Lipase from *Burkholderia cepacia* Lu10-1

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**Abstract:** Even though lipase genes from a variety of microorganism have been cloned and over-expressed, the prospective lipase resources for commercial production and industry application are still limited. In the present study, a lipase from *Burkholderia cepacia* Lu10-1 is heterologously over-expressed in *Escherichia coli* strain BL21(DE3) and purified to homogeneity. The molecular weight of the recombinant lipase from *B. cepacia* Lu10-1 (abbreviated as lipase Lu10-1) is estimated to be about 33 kDa by SDS-PAGE. The lipase Lu10-1 has a priority for the long- chain length substrates. The optimal temperature of lipase Lu10-1 is 60°C and it preserves high thermostability with residual activities of over 80% after 100 h at 60°C or over 60% after 30 h at 70°C. The optimal pH of lipase Lu10-1 is 9.0 and it has broad pH adaptability over a range of 5.0-10.0 retaining 80% activity between pH 6.0 and 9.0 after incubation at 37°C for 24 h. Moreover, the enzymatic activity of lipase Lu10-1 is not obviously affected by several metal ions and it exhibits solid tolerance and stability towards various surfactants and organic solvents. The present study provides the basis for the potential applications of lipase Lu10-1 in related industries.

**Keywords:** Lipase, *Burkholderia cepacia*, *Escherichia coli*, Expression, Characterization

## Introduction

Lipase, or triacylglycerol acylhydrolase (EC 3.1.1.3) can not only hydrolyze glycerol at the oil-water interface and ester bonds of triglycerides, but also catalyze transesterification and ester synthesis. In view of a variety of functions, lipase has commercial values in detergent, food textile, paper, cosmetics, leather tanning, biofuel and bioremediation, etc (Fickers *et al.*, 2011; Garcia-Silvera *et al.*, 2017; Jaeger *et al.*, 1999). Although a lot of lipases can be separated from plants or animals, recently, because of their versatile catalytic capacity, particular biochemical property, high production and simplicity of gene manipulation, more and more research was focused on microbial lipases (Sangeetha *et al.*, 2011; Singh and Mukhopadhyay, 2012).

It has been reported that the lipase from *Burkholderia* (previously *Pseudomonas*) *cepacia* possesses attractive particular interest due to its high thermostability and strong tolerance to organic solvents (Jia *et al.*, 2010;

Mello Bueno *et al.*, 2015; Sasso *et al.*, 2016), therefore, different kinds of *Burkholderia* lipase genes have been cloned and over-expressed in heterogenous or homologous hosts (Jia *et al.*, 2010; Shu *et al.*, 2016; Wang *et al.*, 2009; Xie *et al.*, 2016; Yang *et al.*, 2007). However, the high yield of the active forms in heterogenous host such as *Escherichia coli* has not yet been achieved. The main reason is that most of lipase proteins exist in the cell periplasmic space in the form of inactive inclusion bodies and the refolding procedure is request to obtain the active proteins, which limits the lipase for large-scale industrial application.

In the present study, a lipase from bacterium *B. cepacia* strain Lu 10-1 (GenBank, EF546394) was successfully cloned and over-expressed in *E. coli* as a secreted protein. In addition, the recombinant enzyme was purified to homogeneity and its enzymatic properties were also investigated in detail. This study would provide the basis for industry application of the recombinant lipase Lu10-1.

## Materials and Methods

### Bacterial Strain, Plasmids and Chemicals

A novel strain of *B. cepacia* Lu 10-1 possessing well-established lipase activity was previously isolated and identified by 16S rRNA gene sequencing (GenBank EF546394) in our lab (Ji *et al.*, 2010). The plasmid pMD18T and *E. coli* JM109 were used for gene cloning. The plasmid pET20b(+) and *E. coli* BL21(DE3) were used for heterologous expression in *E. coli*.

### Medium and Culture Conditions

Luria-Bertani (LB) medium, containing peptone 10 g/l, yeast powder 5 g/L, NaCl 10 g/L, pH 7.1, was used for seed medium. The fermentation medium contained glucose 6 g/L, peptone 12 g/L, yeast powder 24 g/L, K<sub>2</sub>HPO<sub>4</sub> 3H<sub>2</sub>O 16.43 g/L, KH<sub>2</sub>PO<sub>4</sub> 2.31 g/L, pH 7.1. *E. coli* cells were grown in LB medium at 37°C and ampicillin was added to the medium with a final concentration of 100 µg/mL.

### Gene Cloning of Lipase Lu10-1

The lipase Lu10-1 was isolated by PCR amplification from the genome of *B. cepacia* Lu10-1 with the forward primer (5'-3': GGAATACCATATGTCCATGGCCGCTGGCTACGCGGCGA) and the reverse primer (5'-3': CATCTCGAGAGAATTCGGTTACACGCCCGCCAGCTTCAGCCG) that introduced the *Nco*I and *Eco*R1 restriction sites at the 5'- and 3'- ends, respectively. The PCR procedure was progressed by 35 successive cycles as follows: denaturation at 94°C for 30 sec, annealing at 56°C for 60 sec and extension at 72°C for 120 sec. Following the PCR, this gene is subsequently ligated into two different vectors, pMD18T-simple and pET20b(+), using *Nco*I and *Eco*R1 sites and the successful insertion of the gene was confirmed by restriction digestion and DNA sequencing. The plasmids with the correct sequence for lipase Lu10-1 were named pMD18T/lip and pET20b/lip, respectively.

### Expression and Purification of Lipase Lu10-1

*E. coli* BL21(DE3) harboring the plasmid pET20b/lip was inoculated into 50 mL of seed medium with 100 µg/mL ampicillin at 200 rpm and 37°C for 8 h. A 5% (v/v) inoculum was inoculated into 100 mL fermentation medium (containing 100 µg/mL ampicillin) at 200 rpm and 30°C. When the optical density at 600 nm (OD<sub>600</sub>) of the culture reached at about 1.5 to 2.0, the inducer Isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added in a final concentration of 0.5 mM. Then, after 18 h of induction, the culture was centrifuged (10,000 × g, 30 min, 4°C) and the supernatant and cells were collected, respectively. The cells were broken by ultrasonic waves. Supernatant and cell fractions (soluble and insoluble cytoplasm) were suspended for protein detection using SDS-PAGE.

70% (w/v) saturated ammonium sulphate solution was added to the supernatant abovementioned and then the solution was kept at 4°C for 12 h. Precipitates were centrifugally collected and dissolved in 100 mL buffer A (20 mM imidazole, 20 mM sodium phosphate and 0.5 M NaCl, pH 7.4). Subsequently, the above solutions were dialyzed by 2 l of equal buffer A for 12 h and employed to a nickel affinity column. The column was pre-equilibrated with buffer A for 2 column volumes and then samples were loaded to bind Ni-NTA with a flow rate of 1 mL/min. Washing elution with buffer A was followed while reaching the UV baseline. A linear gradient from 0 to 500 mM imidazole in buffer A was continued to wash by 2 column volumes. The collected fractions which included *p*NPP lipase activity were assembled and dialyzed by 2 l of buffer B (20 mM Tris-HCl, pH 8.0) at 4°C for 12 h. The purified lipase was finally concentrated by ultrafiltration and stored at -80°C.

### Determination of Bacteria Biomass

The optical density of the culture at 600 nm (OD<sub>600</sub>) during cultivation which represented the bacterial biomass was monitored by a spectrophotometer.

### Lipase Activity Assay

Lipase activity was estimated as previously described (Gricajeva *et al.*, 2016), utilizing *p*-Nitrophenyl palmitate (*p*-NPP) as substrate. The mixture solution (100.0 mL) containing 1 mL of *p*-NPP (10 mM), 4 mL of absolute ethanol and 95 mL of Tris-HCl (50 mM) was prepared and stored at -20°C. A 4 mL above solution was incubated with 0.1 mL of appropriately diluted enzyme for 15 min at 40°C. The reaction was immediately stopped by ice bath.

The activity against *p*-Nitrophenyl butyrate (*p*NPB) with minor revision was determined as previously reported (Chen *et al.*, 2008; Zhang *et al.*, 2010). A final volume of 1 mL mixture solution containing *p*NPB (1 mM) and the assay buffer (50 mM sodium taurodeoxycholate, 10 mM NaCl and 50 mM Tris-HCl, pH 8.0) was incubated 0.02 mL appropriately diluted enzyme for 1 min at 40°C.

The hydrolysis of *p*NPP and *p*NPB for the formation of *p*NP was monitored at 410 nm by a UV-vis spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the substrate and liberated 1 µmol of *p*-nitrophenol (*p*NP) per min at pH 8.0 and 40°C. The experiments and their blank control were repeated in triplicate.

The activity towards triolein or tributyrin was measured as previously reported (Jaeger *et al.*, 1994) with some modifications. Triolein or tributyrin emulsion was prepared by emulsifying triolein/tributyrin and 0.5% (w/v) gum arabic in 25 mM potassium phosphate buffer (pH 9) for 10 min in high-speed tissue crusher. The

reaction solution containing 2.5 mL of 25 mM potassium phosphate buffer (pH 9) and 2 mL of emulsion and enzyme was incubated for 15 min and then finished by adding 7.5 mL of ethanol. The determination of released fatty acids was performed by titration with 2 M NaOH. One unit of lipase activity was defined as the amount of enzyme that released 1  $\mu$ mol of fatty acid per min at pH 9.0 and 40°C. The experiments and their blank control were repeated in triplicate.

#### *Temperature Optimum and Thermostability*

Temperature optima of the recombinant lipase Lu10-1 was surveyed at temperatures ranging from 35 to 80°C. The enzyme activity was determined by pre-incubating the reaction buffer [50 mM sodium taurodeoxycholate, 10 mM NaCl and 20 mM Tris-HCl, pH 9.0] with *p*NPP as substrates at different temperature for 5 min. The determination of enzyme thermostability was performed in Tris-HCl (20 mM, pH 9.0) at the various temperatures (50-80°C) with enzymes. Samples were removed and measured for their residual activity with *p*NPP as substrate at different intervals. The experiments were repeated in triplicate.

#### *pH Optimum and Stability*

pH optima of the recombinant lipase Lu10-1 was investigated at a pH range of 6.0 to 10.0 by using potassium phosphate buffer (pH 6.0-7.0), Tris-HCl buffer (7.0-9.0) and sodium carbonate buffer (pH 9.0-10.0), respectively. pH stability was determined in the following buffers with the concentration of 20 mM: sodium acetate (pH 4.0-6.0), potassium phosphate (pH 6.0-7.0), Tris-HCl (pH 7.0-9.0) and glycine-NaOH (pH 9.0-11.0). The reaction was performed by pre-incubating the enzyme in above buffers at 37°C for 24 h and then the residual activities with *p*NPP as substrate were followed to assay. The experiments were repeated in triplicate.

#### *Effect of Metal Ions on Enzyme Activity*

The effect of metal ions ( $Mn^{2+}$ ,  $Fe^{2+}$ ,  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Zn^{2+}$ ,  $Cu^{2+}$ ,  $Ni^{2+}$  and  $Ba^{2+}$ ) or chelator (EDTA) on the recombinant lipase Lu10-1 activity was performed as follows: 5 nM purified enzyme was pre-incubated with each above metal ion at a concentration of 1mM in Tris-HCl buffer (50 mM, pH 8.0) at 37°C for 30 min and then measured for residual activity against *p*NPP. Enzyme activity without metal ion added was defined as 100%. The experiments were repeated in triplicate.

#### *Effect of Surfactants on Enzyme Activity*

The effects of various surfactants [Triton X-100, Tween 20, Tween 80, SDS and sodium taurodeoxycholate (TDOC)] at different concentrations on the recombinant lipase Lu10-1 were investigated by adding 5 nM purified enzyme to Tris-HCl buffer (50

mM, pH 9) with each surfactant at a concentration of 1 mM or 10 mM and the solution was pre-incubated at 37°C for 30 min and then assayed for residual activity against *p*NPP. Enzyme activity without added surfactants was defined as 100%. The experiments were repeated in triplicate.

#### *Stability in Organic Solvent*

The stabilities of the recombinant lipase Lu10-1 in different organic solvents (methanol, ethanol, isopropyl alcohol, butanol, acetone, n-hexane, benzene, amyl alcohol and dimethyl sulfoxide) were tested in a Tris-HCl buffer (50 mM, pH 9) containing 75% (v/v) of each solvent and 5 nM purified enzyme. After 18 h of incubation at 20°C, aliquots were removed for determination of residual activity against *p*NPP. Enzyme activity without organic solvents was defined as the 100% level. The experiments were repeated in triplicate.

## **Results and Discussion**

### *Cloning, Expression and Purification of Lipase Lu 10-1 in E. coli*

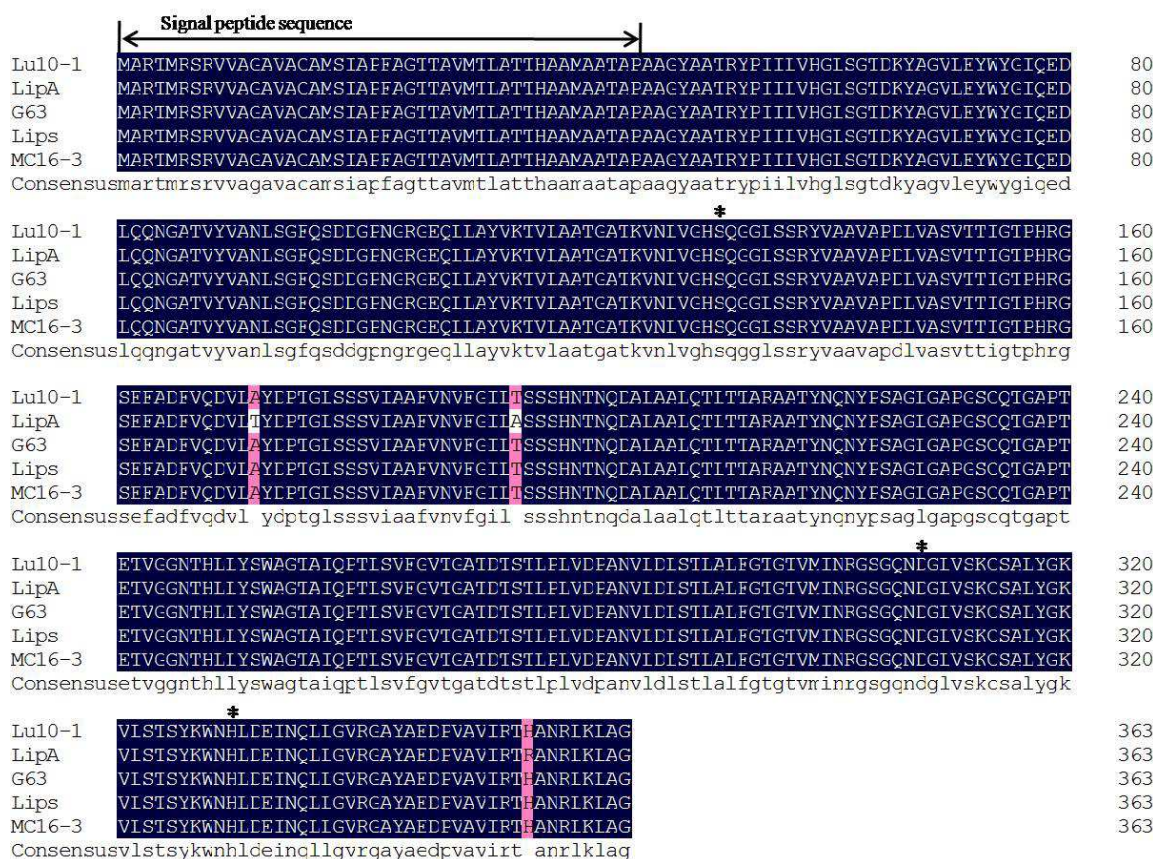
The gene of lipase Lu10-1 excluding the signal peptide fragment of 44 amino acids (Fig. 1) was cloned from the total DNA of *B. cepacia* Lu10-1 and heterologously over-expressed for further characterization. Nucleotide sequence analysis indicated that this lipase gene length was 960 bp for encoding a mature protein consisting of 320 amino acids. Most mature lipase included the conserved penta-peptide motif (Gly-His-Ser-Gln-Gly) with an active serine residue (Ser 87). Additionally, a catalytic center formed by Ser 87, Asp 264 and His 286, as shown in these *Burkholderia* sp. lipases (Kim *et al.*, 1997; Wang *et al.*, 2009). Multiple sequence alignment showed that lipase Lu10-1 shared 99.8% homology of nucleotide sequence and 100% homology of amino acid sequence with that of *Burkholderia* sp. MC16-3 and *B. cepacia* G63 (Fig. 1). However, lipase Lu10-1 showed three residues (172 T/A, 194A/T, 355R/H) difference with that of *B. cepacia* 25416, which might be one of the reasons for exhibiting different biochemical properties.

The cloned gene described above was followed by insertion into the expression vector pET20b(+), encoding an N-terminal signal peptide PelB and a C-terminal His<sub>6</sub> tag to allow the heterologously over-expressed lipase to be secreted. The resulting construct pET20b/lip was expressed in *E. coli* BL21 (DE3). After culturing for 48 h, the amount of lipase produced from the recombinant strain could reach the activity of 19 U/mL for the culture supernatant and 28 U/mL for the cell lysates with *p*-NPP as substrate respectively, meanwhile no enzyme activity

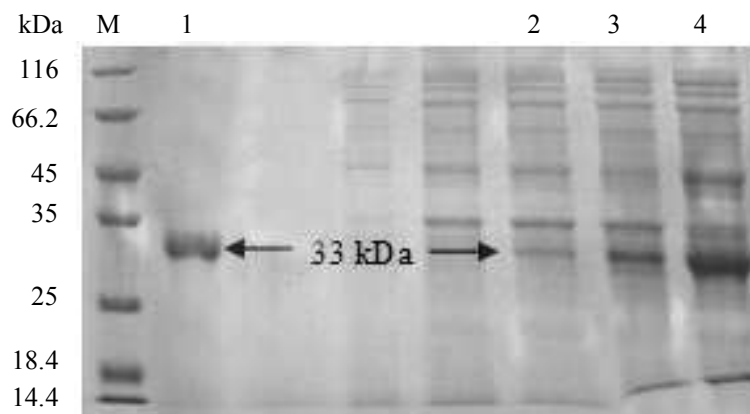
was detected in the control strain [harboring the empty plasmid pET20b(+)]. However, the extracellular activity was much lower than currently used lipases (142 U/mL) from *B. cepacia* (Wang *et al.*, 2009). Due to significant expression of lipase gene concentrated in the form of inclusion bodies in *E. coli* cell pellets (Fig. 2), appropriate strategies to improve the extracellular secretion need further investigation. The extracellular recombinant enzyme was purified to homogeneity simply by one-step nickel affinity chromatography and showed a specific activity of 139 U/mg for lipase Lu10-1 (Table 1). A 4.8-fold purification with 25% recovery was approximately achieved. SDS-PAGE analysis demonstrated that the recombinant lipase Lu10-1 exhibited the apparent molecular mass of ~33 kDa (Fig. 2), which was consistent with lipases from other *Burkholderia* sp. with estimated molecular weights of 29-35 kDa (Dalal *et al.*, 2008; Wang *et al.*, 2009; Xie *et al.*, 2016; Yang *et al.*, 2007; Yao *et al.*, 2013).

### Substrate Specificity

Previous researches have shown that many lipases are provided with broad substrate specificity against soluble ester and insoluble triglycerides (Fickers *et al.*, 2011; Jaeger *et al.*, 1999; Singh and Mukhopadhyay, 2012). Subsequently, the lipase specificity towards the chain length of esters was considered using *p*-nitrophenyl-fatty acyl esters and triglycerides (Table 2). For lipase Lu10-1, its activity against the C16 *p*-nitrophenyl-fatty acid ester *p*NPP was significantly increased by 1.78 fold than that towards the corresponding C4 ester *p*NPB. Similarly, its activity against the C18 ester triolein was also improved by 1.4 fold than that of the corresponding C4 triglyceride tributyrin. Therefore, a trend of preferential specificity towards longer chain length substrates is obviously evident. Most lipases, especially from *Burkholderia* sp. prefer medium- or long-chain fatty acid esters in previous study (Patel *et al.*, 2014; Wang *et al.*, 2009; Xie *et al.*, 2016; Yao *et al.*, 2013), which was consistent with our findings.



**Fig. 1:** Multiple alignment of amino acid sequences of lipase Lu10-1 with several other lipases. Abbreviations and accession numbers of lipases are as follows: lipase Lu10-1 from *B. cepacia* Lu10-1, lipase LipA from *B. cepacia* ATCC 25416 (accession no. ADT80785.1), lipase G63 from *B. cepacia* G63 (accession no. ABN09945.1), lipase MC16-3 from *Burkholderia* sp. MC16-3 (accession no. AAV34203.1), lipase Lips from *B. cepacia* (accession no. WP027791104.1). active-site residues are indicated by stars (\*)



**Fig. 2:** SDS-PAGE analysis of over-expressed and purified lipase Lu10-1. Lane M, molecular mass marker; lane 1, purified lipase Lu10-1; lane 2, culture supernatant of *E. coli* BL21(DE3)/pET20b/lip; lane 3, cell lysate of *E. coli* BL21(DE3)/pET20b/lip; lane 4, cell inclusion body of *E. coli* BL21(DE3)/pET20b/lip

**Table 1:** Purification of recombinant lipase Lu10-1 from *E. coli* BL21(DE3)

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg protein)	Yield (%)	Purification (fold)
Crude extract	664	19511	29	100.0	1.0
Ammonium sulfate fraction	182	11629	64	60	2.2
Ni-Sepharose affinity purification	35	4852	139	25	4.8

**Note:** The enzymatic activity was assayed using *p*NPP as substrate

**Table 2:** Specificity of lipase Lu10-1 towards the acyl chain length of different esters

Substrate	Specificity activity (U/mg)
<i>p</i> NPB	78±11
<i>p</i> NPP	139±8
tributyrin	91±5
triolein	124±4

**Note:** Specificity of lipase Lu10-1 was determined at its optimal temperature of 60°C. Values are means ± SD (n = 3)

### Effect of Temperature and pH on Activity and Stability of Lipase Lu10-1

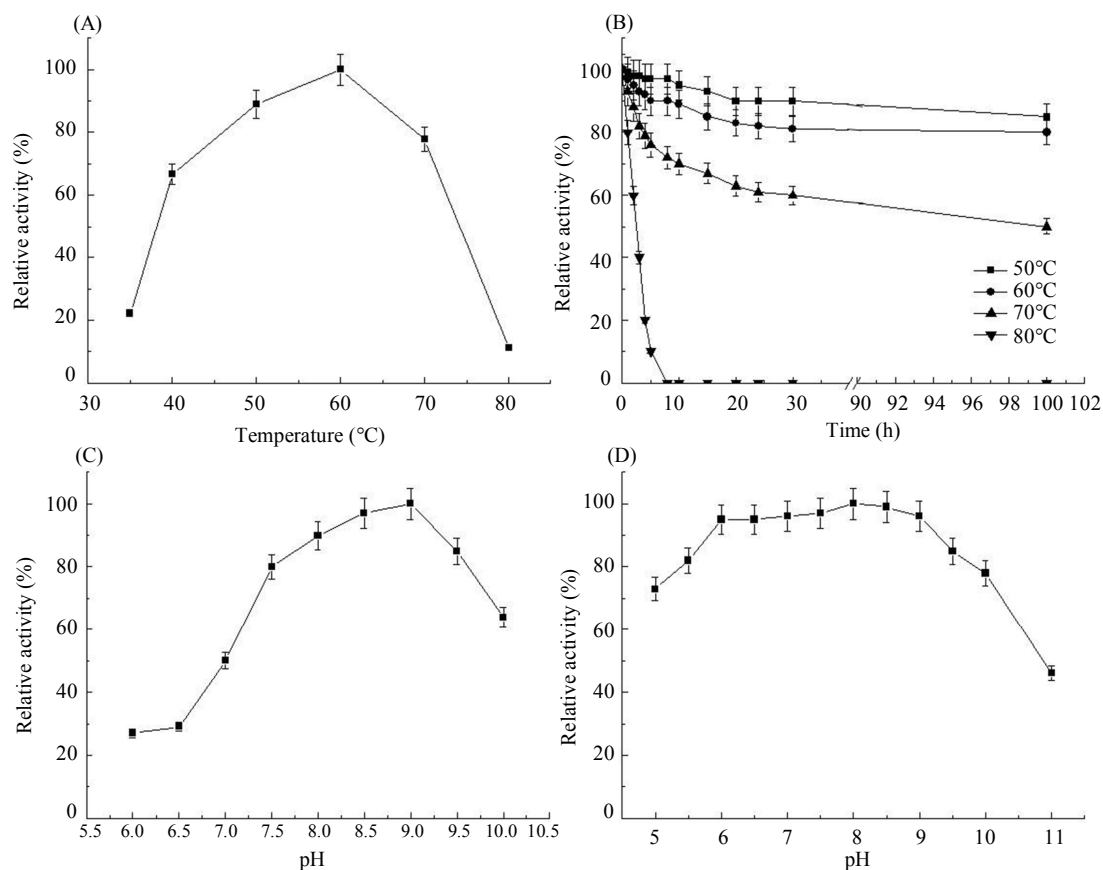
Generally in biodiesel production or other high temperature industries, high temperature (>40°C) could increase the diffusion and miscibility in different phases and thereby improve the conversion efficiency. In this study, the influence of temperature on the enzyme activity was investigated with a range from 35 to 80°C at pH 8.0 (Fig. 3). The optimum temperature curve of lipase Lu10-1 exhibited that the enzyme activity improved followed with increasing temperature from 35 to 60°C and decreased from 60 to 80°C and it retained nearly 80% residual activity at 70°C (Fig. 3A). The activity at the optimum temperature of 60°C was 5-fold higher than that at 35°C. However, most lipases from fungi or bacterium had an optimum temperature at 40-45°C (Dalal *et al.*, 2008; Fickers *et al.*, 2011; Sun *et al.*, 2016; Xie *et al.*, 2016). Of course, a small number of lipases could reach an optimal temperature of 60°C (Wang *et al.*, 2009; Yao *et al.*, 2013). The thermostable experiment showed that lipase Lu10-1

preserved high thermostability, with residual activities of over 80% after 100 h at 60°C or over 60% after 30 h at 70°C (Fig. 3B), while lipases reported from other *B. cepacia* strains, such as ATCC25609 or RQ3 possessed lower stability below 50°C with a half-life of less than 1 h (Dalal *et al.*, 2008; Xie *et al.*, 2016).

The pH optimum and stability of lipase Lu10-1 were measured at a pH range of 5.0-11.0 (Fig. 3). The lipase Lu10-1 displayed comparatively high activity under alkaline conditions and performed an optimum pH of 9.0 (Fig. 3C). The enzyme was considerably stable with a broad pH range from 5.0 to 10.0 and retained more than 80% of its initial activity at pH 6.0 - 9.0 after incubation at 37°C for 24 h (Fig. 3D). Most microbial lipases displayed narrow pH stabilities, for example, the lipases from *B. cepacia* ATCC25609 and LP08 were merely stable at pH 9.0-10.0 and pH 8.0-9.0, respectively (Dalal *et al.*, 2008; Wang *et al.*, 2009). Obviously, lipase Lu10-1 showed a stable performance across a wide pH range, which would be advantageous to its application in complex and diverse industrial environments.

### Metal ion Requirement

According to previous reports, most lipases from *B. cepacia* and other *Burkholder sp.* required the presence of metal ions to fully exert their activity (Kim *et al.*, 2001). The requirement of metal cofactor for lipase Lu10-1 was performed by incubating the enzyme with the metal ions or metal chelator EDTA and then assayed for activity against *p*NPP (Table 3).



**Fig. 3:** Effects of pH and temperature on activity and stability of lipase Lu10-1. over Temperature optimum. The samples were determined in Tris-HCl (pH 9.0). The activity of lipase Lu10-1 at 60°C as 100%. (B) Thermostability. The stability of the enzymes were performed in Tris-HCl (pH 9.0) at 50°C (■), 60°C (●), 70°C (▲) and 80 °C (▼). The activity of lipase Lu10-1 without heat treated was defined as 100%. (C) pH optimum. The samples were determined in the following buffer-potassium phosphate (pH 6.0-7.0), Tris-HCl (7.0-9.0) and sodium carbonate (pH 9.0-10.0). The activity of lipase Lu10-1 at pH 9.0 was defined as 100%. (D) pH stability. The enzyme activity was determined after incubation 24 h at 37°C in the following buffers- sodium acetate (pH 4.0-6.0), potassium phosphate (pH 6.0-7.0), Tris-HCl (pH 7.0-9.0) and glycine-NaOH (pH 9.0-11.0). The activity of lipase Lu10-1 at pH 9.0 was defined as 100%. All activity were performed in triplicate. Error bars correspond to the standard deviation of three independent determinations

**Table 3:** Effect of metal ions and metal chelator on lipase Lu10-1 activity

Metal ions/Metal chelator	Relative activity (%)	Metal ions/Metal chelator	Relative activity (%)
Control	100±5	Zn <sup>2+</sup>	85±5
Mn <sup>2+</sup>	67±4	Cu <sup>2+</sup>	96±5
Fe <sup>2+</sup>	78±5	Ni <sup>2+</sup>	101±5
Mg <sup>2+</sup>	94±4	Ba <sup>2+</sup>	92±3
Ca <sup>2+</sup>	118±7	EDTA	89±4

**Note:** The lipase Lu10-1 was pre-incubated with metal ions or metal chelator (1 mM) at 37°C for 30 min and then assayed for lipase activity using pNPP as substrate. Values are means ± SD (n = 3)

Among the tested metal ions, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup> and Ba<sup>2+</sup> did not exhibit a significant influence on the enzyme activity, whereas Mn<sup>2+</sup>, Fe<sup>2+</sup> and Zn<sup>2+</sup> with remaining only 67, 78 and 85% of activities, respectively, showed a medium inhibitory effect. According to previous report, the calcium-binding site was well conserved (Kim *et al.*, 2001) in the crystal

structures of lipases from *B. cepacia* and other species. Many lipases have been reported to display significantly enhanced activity (increased 2-3 fold) in the presence of Ca<sup>2+</sup> (Wang *et al.*, 2009; Xie *et al.*, 2016), but this stimulation by Ca<sup>2+</sup> was not obviously found in our study. The metal chelator EDTA exhibited a little effect on the enzyme activity, which was consistent with the

results of lipase from *Pseudomonas stutzeri* LC2-8 (Cao *et al.*, 2012) and *Burkholderia ambifaria* YCJ01 (Yao *et al.*, 2013). It might be suggesting that lipase Lu10-1 did not require divalent cations for its activity.

### Lipase Stability Towards Surfactants

Application of industrial enzymes often involves relatively harsh conditions such as the presence of surfactants and organic solvents. The activity of lipase Lu10-1 was tested in the presence of the nonionic surfactants Triton X-100, Tween 20, Tween 80 and anionic surfactants SDS and TDOC (Table 4). At concentrations of 1 mM and 10 mM, Triton X-100, Tween 20, Tween 80 and SDS did not significantly reduce enzyme activity. The effect of TDOC was only one exception because it stimulated the activity of lipase Lu10-1 with a 9% increase at 1 mM and a 29% increase at 10 mM after incubation for 30 min at 37°C. TDOC is an anionic surfactant with a bulky side chain which might bind to the hydrophobic sites of proteins preventing their aggregation and rendering them more stable (Creveld *et al.*, 2001).

### Organic Solvent Stability of Lipase Lu10-1

Lipases have been reported to show esterification and transesterification activity and have potential use in bioconversion industry (Jaeger *et al.*, 1999; Sasso *et al.*, 2016; Yang *et al.*, 2007). Such applications often involve the use of organic phase, therefore, the activity and stability in organic solvents are identified as novel feature for lipase. Influences of different organic solvents with a concentration of 75% on the stability of lipase Lu10-1 were studied by incubating enzyme solution after incubated for 18 h at 20°C. As shown in Table 5, there was not significant effect on the activity of lipase Lu10-1 in methanol, ethanol, n-hexane, benzene and dimethyl sulfoxide. Among of all the tested organic solvents, lipase Lu10-1 exhibited a high tolerance in amyl alcohol, with the remaining activity of 92%. However, the endurance of lipase Lu10-1 to isopropyl alcohol, butanol and acetone is restricted to some extent and the remaining activity was 57, 43 and 75%, respectively. Despite the reported solvent stability for other lipases with a usual concentration for organic solvents of only 50% or less (Wang *et al.*, 2009; Yang *et al.*, 2007) and most lipases were still inhibited by organic solvents like ethanol, methanol and isopropanol. Result from our study showed that lipase Lu10-1 is able to be tolerate to a higher concentration (75%) of organic solvent used in biodiesel production. The structure-function relationship about the solvent tolerance of this lipase could be provided for further research. The obvious stability of lipase Lu10-1 towards organic solvents made it ideal for applying as a biocatalyst in non-aqueous media.

**Table 4:** Effect of surfactants on lipase Lu10-1 activity

Surfactants Concentration	Relative activity (%)	
	1 mM	10 mM
Triton X-100	88±4	84±4
Tween 20	60±3	53±3
Tween 80	74±4	72±4
SDS	85±4	84±4
TDOC	109±5	129±6

**Note:** The lipase Lu10-1 was pre-incubated with the surfactant at 37°C for 30 min and then assayed for lipase activity using pNPP as substrate. Values are means ± SD (n = 3)

**Table 5:** Stability of lipase Lu10-1 in organic solvents

Organic solvents	Relative activity (%)	Organic solvents	Relative activity (%)
Control	100±3	Acetone	75±4
Methanol	84±4	n-Hexane	85±4
Ethanol	89±4	Benzene	82±4
Isopropyl alcohol	57±3	Amyl alcohol	92±3
Butanol	43±2	Dimethyl sulfoxide	89±4

**Note:** The lipase Lu10-1 was preincubated with 75% (v/v) of organic solvents in assay buffer at 20°C for 18 h. Aliquots were removed for determination of residual activity. Values are means ± SD (n = 3)

## Conclusion

In summary, the lipase from *B. cepacia* Lu10-1 was cloned, heterologously over-expressed and its biochemical properties were investigated in detail. The purified recombinant lipase Lu 10-1 possessed maximum activity at 60°C and pH 9.0, had excellent thermostability (remaining over 80% activity after 100 h at 60°C or over 60% after 30 h at 70°C) and broad pH adaptability (retaining 80% activity between pH 6.0 and 9.0 after incubation at 37°C for 24 h) and was highly tolerant and stable to kinds of surfactants and organic solvents. Thus, the lipase Lu10-1 with above distinct properties possesses development prospects in basic research and various industrial applications.

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## Author's Contributions

**Yao Zhang:** Has contributed in a whole experiment, data analysis and publication.

**Lu Wang:** Has assisted in the experiment.

**Yuanda Song:** Has reviewed and revised the manuscript.

## Ethics

All authors read and approved the final version of this manuscript. There are not any ethical issues to declare that could arise after the publication of this manuscript.

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