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Isolation and Characterization of a Novel Bacterium *Burkholderia gladioli* Bsp-1 Producing Alkaline Lipase

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Active lipase-producing bacterium *Burkholderia gladioli* Bps-1 was rapidly isolated using a modified trypan blue and tetracycline, ampicillin plate. The electro-phoretically pure enzyme was obtained by purification using ethanol precipitation, ion-exchange chromatography, and gel filtration chromatography. The molecular weight was 34.6 kDa and the specific activity was determined to be 443.9 U/mg. The purified lipase showed the highest activity after hydrolysis with p-NPC$_{16}$ at a pH of 8.5 and 50°C, and the K_m, k_{cat}, and k_{cat}/K_m values were 1.05 mM, 292.95 s^{-1} and 279 s^{-1}mM^{-1}, respectively. The lipase was highly stable at 7.5 ≤ pH ≤ 10.0. K^+ and Na^+ exerted activation effects on the lipase which had favorable tolerance to short-chain alcohols with its residual enzyme activity being 110% after being maintained in 30% ethanol for 1 h. The results demonstrated that the lipase produced by the strain *B. gladioli* Bps-1 has high enzyme activity and is an alkaline lipase. The lipase has promising chemical properties for a range of applications in the food-processing and detergent industries, and has particularly high potential for use in the manufacture of biodiesel.

Keywords: *Burkholderia gladioli* Bps-1, alkaline lipase, purification, biocatalysis

Introduction

Lipase (E.C. 3.1.3) is a special ester hydrolase and biocatalyst that has multiple catalytic functions in the hydrolysis, acidolysis, alcoholysis, ammonolysis and transesterification reactions. Lipases also have wide-ranging applications in the food processing, paper-making, detergent, and pharmaceutical industries as well. As important metabolic enzymes in organisms, they exist widely in animals, plants and microorganisms. Lipases generally control multiple physiological processes including digestive absorption of organics and metabolism of fat and lipoproteins [1, 2].

Lipases from microorganisms are the most widely investigated in comparison to those from animals and plants [3]. Extracellular lipases exhibit different properties produced by a variety of microorganisms and are involved in multiple reaction types including ester synthesis, transesterification, ammonolysis and alcoholysis [4]. These lipases exhibit properties including low substrate requirements, high activity in mild conditions, lack of unnecessary coenzymes and low energy consumption. They have also been widely applied in multiple fields including food processing, textiles, chemical engineering, detergents, bio-pharmaceuticals and high-molecular polymers [5].

Reports have shown that lipase-producing bacteria comprise approximately 28 genera, including *Bacillus*, *Staphylococcus*, *Burkholderia*, *Acinetobacter*, *Pseudomonas*, *Saccharomycetes* and *Actinomycetes* and a total of 65 fungal genera have been reported to produce lipase [6]. Lipases produced by bacteria are active in the catalysis of most reaction types, have the highest activity, and are most stable in the organic phase [7].

According to homology of rRNA, Yabuuchi *et al*. [8]

partitioned seven kinds of *Pseudomonas cepacia* into new *Burkholderia* sp., which belong to the subdivision of the phylum *Proteobacteria*. Lipases produced by these bacteria have various advantageous properties including favorable temperature stability, tolerance to organic solvents and strict enantio-selectivity. Currently, a growing number of lipases produced by *Burkholderia* sp. are being reported.

Yang [9] acquired a new type of lipase, SL-4, produced by *Burkholderia ubonensis*, which is a new member of subfamily I.2 lipases with a molecular weight of 33 kDa and an optimum pH and temperature of 8.5 and 65°C, respectively. The lipase from *Burkholderia* sp. EQ3 purified by Pakpimol shows maximum activity at a pH of 7.0~7.5 and a temperature of 30°C. It could be reserved for 1 h at pH of 5.0~8.0 with the temperature ranging between 30°C~55°C. The purified lipase EQ3 still exhibited more than 80% activity in 25% isopropanol [10].

Xie [11] isolated the new bacterium *Burkholderia cepacia* RQ3 using polar and non-polar organic solvents (DMSO and methylbenzene) as a screening stress. The bacterium was resistant to organic solvents and the lipase RQ3 had an optimum pH and temperature of 9.0 and 40°C, respectively, and showed favorable stability across a wide pH range (6.0~10.0) and at temperatures lower than 50°C. In addition, a broader working pH and higher thermostability of the lipase were achieved by immobilization [12]. Wang [13] used Fe_3O_4@chitosan nanoparticles for covalent immobilization of lipase from *Thermomyces lanuginosus* by chemical conjugation after electrostatic entrapment (CCEE), and the immobilized lipase retained 70% of its initial activity after ten cycles. Xu [14] reported that CLEAs of lipase prepared by using p-benzoquinone as a cross-linking agent could retain 75.18% of the initial activity of the biocatalyst.

In the present study, *Burkholderia* sp. Bps-1 was isolated by addition of small amounts of antibiotics to the modified trypan blue and tetracycline, ampicillin (TB-TA) plate considering the essential metabolite and the drug resistance of *Burkholderia* sp. The strain was identified as *Burkholderia gladioli* through 16S rDNA sequencing and the Microlog Microbial Identification System. The alkaline lipase produced by the isolate Bps-1 was purified through ethanol precipitation, anion exchange chromatography and gel filtration chromatography. Chemical characterization of the purified Bps-1 lipase showed that the molecular weight, optimum pH and temperature were 34.6 kDa, 8.5 and 50°C, respectively. The lipase Bps-1 exhibited high stability at pH of 7.5 to 10.0 and a favorable tolerance to short-chain alcohols.

Materials and Methods

Specimens

Putrid onion specimens were obtained from a supermarket in Nanning, the Guangxi Zhuang Autonomous Region, China.

Culture Medium

Sterile TB-TA directional screening plate composed of glucose (2 g/l), L-asparagine (1.0 g/l), $NaHCO_3$ (0.5 g/l), KH_2PO_4 (0.25 g/l), $MgSO_4{\cdot}7H_2O$ (0.05 g/l), trypan blue (0.05 g/l), tetracycline (0.01 g/l), ampicillin (0.15 g/l) and agar powder (15 g/l) was used.

The identification plate of Rhodamine B comprised peptone (5 g/l), yeast extract (3 g/l), NaCl (8 g/l), 4 ml Rhodamine B solution (0.1%, w/v) and 10 ml soybean oil emulsion. The soybean oil emulsion was prepared by mixing 3 ml soya-bean oil with 9 ml polyvinyl alcohol (2%, w/v) for 10 min using an emulsifying machine. After settling for 5 min, emulsification continued until delamination disappeared.

Culture medium of seed solution: LB culture medium was made from Tryptone 10 g/l, yeast extract 5 g/l, NaCl 10 g/l.

Fermentation medium was composed of 1.0% (v/v) saccharose, 0.25% (v/v) palm oil, 1.5% (w/v) peptone, 0.2% (w/v) KH_2PO_4 and 0.05% (w/v) $MgSO_4{\cdot}7H_2O$.

Isolation and Identification of *Burkholderia* sp.

Exactly, 5 g of shredded putrid onion was suspended into 100 ml of normal saline and the solution was spread on TB-TA plate at a concentration 10^{-3}-10^{-4} times that of the suspension liquid obtained by gradient dilution. The colonies on the plates with the modified TB-TA medium were then streaked onto the identification plate of Rhodamine B for lipolytic bacteria and cultured at 30°C for 2 days. A single strain was then obtained by purifying the bacterial colonies with a ratio of the radius of a transparent circle to the radius of colony greater than 2 through plate streaking. The isolated strain was morphologically characterized and identified using the Microlog Microbial Identification System (Biolog, USA) and 16S rDNA sequential analysis.

Culture Conditions for Producing Lipase

Some colonies from an LB agar plate were inoculated into the fermentation medium and incubated at 30°C until the log phase of the culture was obtained. Exactly, 1% (v/v) of this culture was inoculated into the fermentation medium and incubated for 72 h at 30°C. Subsequently, the broth was centrifuged for 10 min at 8,000 ×*g* and the supernatant was collected for the use as a crude enzyme specimen.

Lipase Activity Assay

Solution A: 16.5 mmol/l ρ-nitrophenol palmitate ($pNPC_{16}$) solution in isopropanol. Solution B: Tris-HCl buffer solution (pH = 9.0) of 0.05 mol/l using 2% (w/v) Triton X-100 and 0.1% (w/v) Arabic gum.

The reaction system was described as follows: 45 μl of solution A was mixed with 405 μl of solution B after pre-heating at 50°C for 5 min, the diluted liquid enzyme sample was reacted for 10 min and a spectrophotometer method was used to measure the amount of liberated $pNPC_{16}$ at a wavelength of 405 nm. The reaction was stopped by adding 500 μl of 10% (w/v) trichloroacetic acid: 1 ml of 0.5 M Na_2CO_3 was then added to adjust the pH so that the reaction system was alkaline. The lipase activity unit U was defined as the amount of lipase consumed by the decomposition of $pNPC_{16}$ for the production of 1 μmol of nitrophenol per minute. All experiments were performed in triplicate [15−17].

Purification of Lipase

The crude enzyme was freeze-dried in a vacuum freeze-drying system. Exactly, 6 g of the freeze-dried crude enzyme was dissolved in 2 ml of 0.05 mol/l piperazine buffer solution at a pH of 9.7. The mixture was precipitated by adding ethanol to a final concentration of 60% (w/v), after which the mixture was centrifuged for 10 min at 8,000 ×*g* to remove unspecific proteins. The target protein was precipitated using ethanol with a final concentration of 95% (v/v) followed by centrifugation at 8,000 ×*g* for 10 min. Precipitations were dissolved in 0.05 mol/l piperazine buffer solution at pH of 9.7 to obtain the preliminarily purified enzyme which was further purified through HiTrap Q (5 ml) and Superdex 75 gel filtration columns (100 × 1.2 cm).

Effect of Temperature on Activity and Stability

The relative lipase activity was measured at different temperatures (30~75°C) to obtain the optimum reaction temperature. Residual activity was tested after the diluted enzyme was maintained for 1 hour at temperatures ranging from 25 to 85°C. The thermal stability of the lipase was analyzed by comparison with the untreated control enzyme.

Effect of pH on Activity and Stability

The relative lipase activity was measured and the optimum pH for reaction was determined after the substrate–$pNPC_{16}$ was dissolved in buffer solution at different pH values (2.2−10.0). After the lipase was diluted using the buffer solution at a pH ranging from 2.2 to 12.0, it was maintained for 24 h at 25°C and then the residual activity was measured. The pH stability of lipases was analyzed by comparison with the untreated control enzyme without the addition of buffer.

Effect of Organic Solvents on Lipase Activity

The lipase was diluted using Tris-HCl buffer solution at a pH of 8.5. n-heptane (0.2), n-hexane (0.06), cyclohexane (0.1), methanol (5.1), ethanol (5.2), isopropanol (3.9), ethyl acetate (4.4), acetone (5.1) and methylbenzene (2.4) were separately added so that the final concentration of organic solvent was 30% (v/v). After the mixture was maintained for 1 hour at an oscillation rate of 300 r/min at 50°C, the relative lipase activity was determined. By comparing the enzyme with and without adding the organic solvent, the effect of organic solvent on the lipase was calculated.

Effect of Metallic Ions and Protease Inhibitor on Enzyme activity

The lipase was maintained in a solution containing various metallic ions, β-mercaptoethanol, EDTA, SDS and DDT at the final concentration of 10 mmol/l after being diluted using Tris-HCl buffer solution at a pH of 8.5. After the mixture was stored for 1 h at an oscillation rate of 300 r/min at 30°C, the relative lipase activity was obtained. The effects of metallic ions and protein inhibitors on the lipase were investigated by comparing the enzyme with the control without adding organic solvents.

Substrate Specificity and Kinetic Analysis

Certain amounts of p-nitrophenol fatty acid esters (C_2, C_4, C_8, C_{10}, C_{12}, C_{14}, C_{16}, and C_{18}) with different carbon-chain lengths were weighed as substrates with a final concentration of 16.5 mmol/l. The relative activities of the lipase on various substrates were detected at a pH of 8.5 and temperature of 50°C. Data were normalized as a percentage of the largest relative lipase activity [18].

The four substrates (C_{12}, C_{14}, C_{16}, and C_{18}) with the highest hydrolytic activities were taken and the activities of the lipase were measured when the concentrations of the substrates were 0.2, 0.3, 0.4, 0.5, 0.625, 1.0, 2.0, and 5.0 mmol/l. Using non-linear regression (OriginPro8.5), the kinetic constants (K_m and V_{max}) and the catalytic constants (k_{cat} and k_{cat}/K_m) of the lipase were calculated.

Results

Screening and Identification of Strains

At present, research into *Burkholderia* sp. is increasingly widespread and the *Burkholderia cepacia* complex (BCC) has been most intensively studied [19]. Numerous methods for isolating bacterial genera are known, amongst which the most rapid is the plate screening method, which exhibits a directional screening rate of up to 90% using special metabolites and drug resistance. Owing to BCC belonging to *Burkholderia* sp., the growth conditions, physiological characterization and drug resistance of the two substances show many similarities. Ampicillin and nystatin (TB-TA plate) with a certain concentration were added into the selected plate TB-T of BCC and the proportion of L-asparagines (only nitrogen source) was improved. On this basis, the culture medium was named TB-TA, and applied to directionally isolate the *Burkholderia* sp. strain. Bacterial colonies grew up on the plate after culturing the strain for 3−4 days at 30°C.

The developed bacterial colonies were dripped on the Rhodamine B plate for identification as shown in Fig. 1, showing that the bacterial colonies with transparent circles

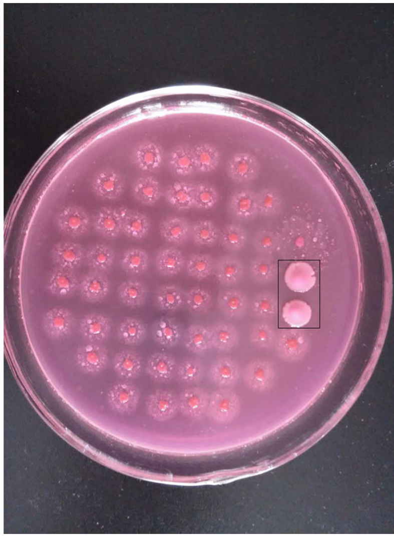

Fig. 1. Production of lipases by *Burkholderia* sp. complex strains on the selective screening medium for lipase activity.
Only two colonies (marked with a black frame) did not possess sufficient activity to hydrolyze olive oil on the olive oil-rhodamine B plate.

accounted for more than 95%. The single strain was obtained after the bacterial colonies with a radius ratio of the transparent circle to colony of greater than 2 were purified through plate streaking. The activities of the lipase of 13 strains subjected to the liquid culture were measured and the strain with the highest activity was named Bps-1. The strain was morphologically characterized as a Gram-negative bacterium, which forms faint yellow, round, smooth and moist bacterial colonies with regular sides on LB medium and produces xanthein after growth for 2 days. The bacterium is aerobic and motile, has rod-like cells without spores and grows at 30−35°C and pH of 5.0−8.0 (optimal pH of 6.5−7.5). The bacterium was identified as *Burkholderia 25gladioli* using the Biolog system (the value of SIM was 0.575 after being cultured for 24 h).

The length of 16S rDNA gene of the bacterium Bps-1 was determined to be 1,500 bp by PCR amplification. Sequencing showed that the sequence had 1,493 bp and was deposited in NCBI after biting off the chimera sequence (accession number of the sequence: MF618254). The comparison results of BLAST revealed that the sequence exhibited the highest similarity with 16S rDNA sequence of *Burkholderia gladioli*. Using a phylogenetic tree (shown in Fig. 2) designed using MEGA6.0, it can be seen that the strain belonged to the same population as the *Burkholderia gladioli* strain GRBB 15043. Through the analysis of the morphological features and identification using the Biolog system, it was verified that the strain was *Burkholderia gladioli*. In summary, the rapid isolation of the *Burkholderia* sp. strain was achieved using a selection plate of TB-TA.

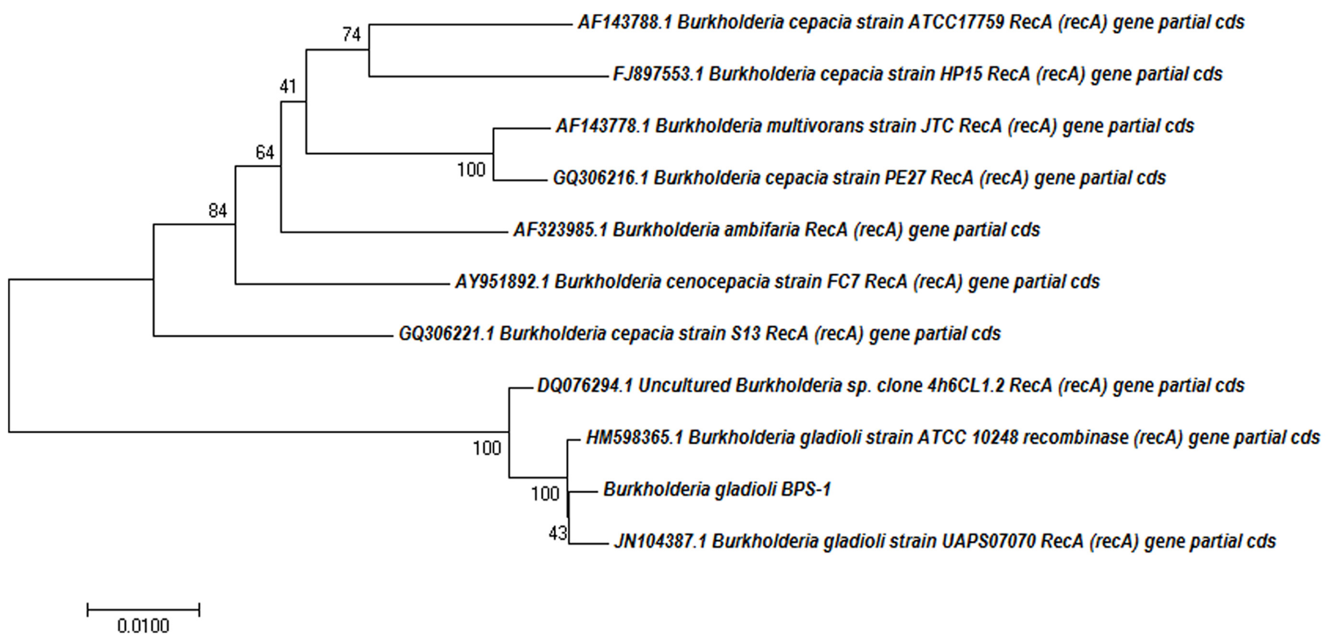

Fig. 2. Phylogenetic tree based on the 16S rDNA gene sequence of isolated *B. gladioli* Bps-1 and other related species.
Levels of bootstrap support (percent, n = 1,000) were indicated at nodes. The scale bar represents 0.001 nucleotide substitutions per position.

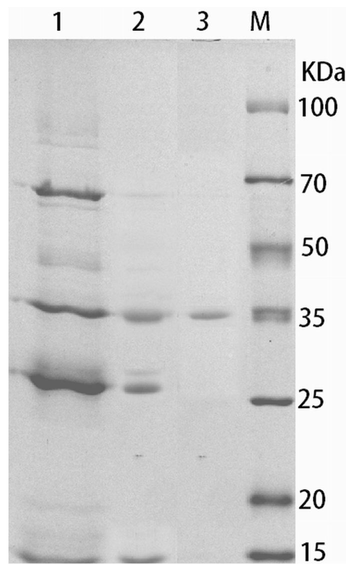

Fig. 3. SDS-PAGE of lipase *B. gladioli* Bps-1.

1: Protein specimen through ethanol precipitation; 2: specimen purified through HiTrap Q; 3: specimen purified through Superdex 75; M: molecular weight markers.

Protein Purification and Measurement of Molecular Weights

The lipase specimens were obtained from the fermentation supernatant of *B. gladioli* Bps-1 and were purified through freeze-drying, ethanol precipitation, HiTrap Q, and Superdex 75. The purified lipase was a single band product with an apparent molecular mass of 34.6 kDa according to SDS-PAGE (Fig. 3). The yield of the lipase was 0.029% after purification (purification fold: 4.57) through the above steps, and the specific activity of the purified lipase in the hydrolysis of $pNPC_{16}$ was 443.90 U/mg (as shown in Table 1).

Effect of Temperature on Activity and Thermal Stability

The optimum temperature and temperature stability of the lipase are shown in Fig. 4A. The optimal reaction temperature of the lipase was 50°C and the purified lipase exhibited favorable thermal stability at temperatures between 25–70°C. The residual activity comprised 50% of the primary activity after the lipase was treated for 1 h at 70°C. It has been reported that lipases produced by Gram-negative bacteria showed unsatisfactory thermal stability. For example, the lipase EQ3 was active at 30~55°C and reached a peak at 30°C. The enzyme activity at 55°C still accounted for 80% of that at 30°C while it significantly decreased at 65°C. The extracellular lipases from *Pseudomonas fluorescens* MTCC 2421 exhibited enzyme activity at 35 to

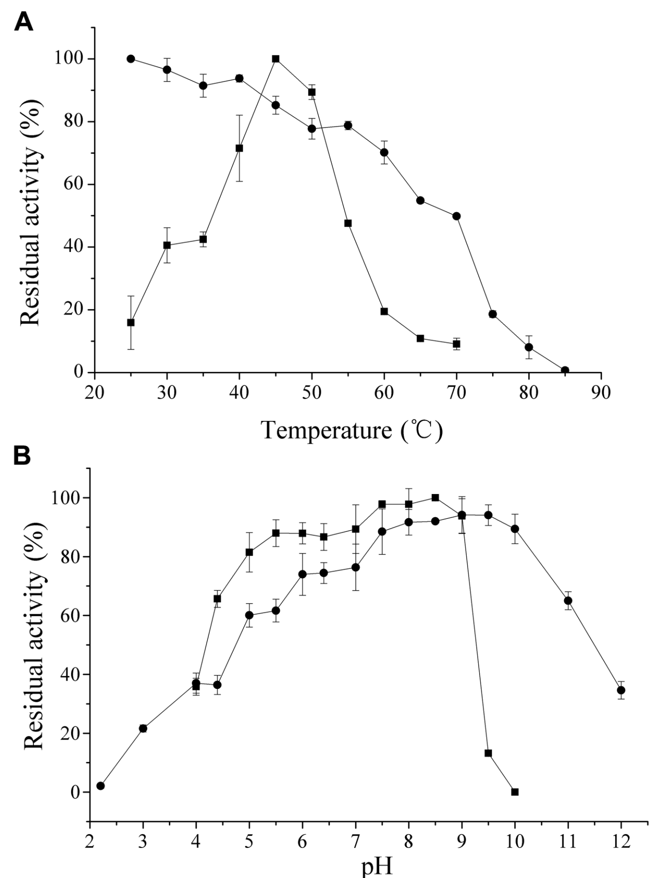

Fig. 4. Effect of temperature and pH on the activity (square) and stability (circles) of lipase Bps-1.

(**A**) The effects of temperature on the activity (square) and stability (circles) of lipase Bps-1. (**B**) The effect of pH on the activity (square) and stability (circles) of lipase from Bps-1.

Table 1. Summary of the purification of lipase from *B. gladioli* Bps-1.

	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification (fold)	Yield (%)
Crude enzyme	6,000	582,680.2	97.11	1	100
Ethanol precipitation	567	58,707.18	103.54	1.07	10.1
HiTrap Q	1.36	357.99	263.23	2.71	0.061
Superdex 75	0.38	168.68	443.90	4.57	0.029

50°C, which increased to a maximum at 40°C, whilst at 50°C decreased by 44% [20].

In addition, the lipase from *Burkholderia* sp. HY-10 only maintained 50% activity after being cultured for 30 min at 60°C [21]. As the stability of enzymes at higher temperatures could greatly increase reaction rates, the thermal stability of Bps-1 lipase was an ideal characteristic.

Effect of pH on Activity and Stability

The optimum pH and pH stability of the purified lipase are illustrated in Fig. 4B, and as shown in the figure, the optimum pH of the lipase was 8.5. The purified lipase exhibited strong stability in an alkaline environment, particularly across a pH range from 7.5 to 10.0, and still had more than 85% residual activity after being maintained for 24 h at pH 10, indicating that the enzyme was an alkaline lipase. Reports have shown that most lipases from *Burkholderia* sp. presented the optimum activity under neutral and alkaline conditions. For instance, the optimum pH of the lipase from *Burkholderia* sp. EQ3 was in the range of 7.0−7.5 with the lipase having greater than 80% activity at the pH of 6.0−8.0 [10]. In contrast, the lipase from *B. cepacia* LP08 exhibited activity across a broader pH range of 5.0 -10.6 and reached maximum activity peak at pH 9.0 [22]. Additionally, the lipase from *B. multivorans* PSU-AH130 could retain more than 80% activity at a pH of 7.5−10.0 [23]. This may be because the structures of the *Burkholderia* sp. producing lipases were more suitable for approaching the oil-water interface in an alkaline environment and then opening the cover which impeded the active sites and so the hydrolysis energy declined. As the key to applying lipases in various technological processes such as detergent and leather manufacturing lies in the stability of the enzymes in alkaline environments, Bps-1 is seen as an ideal enzyme for industrial applications.

Effect of Organic Solvents on Enzyme Activity

Lipases show high catalytic activities in organic solvents and organic solvent-bearing environments. The yield of biodiesel can be significantly improved using lipases that are tolerant to organic solvents, particularly methanol and ethanol. Exploring the tolerance of lipases to organic solvents is an important area of research. In this study, the influences of nine polar and non-polar organic solvents (polar indices 0.06~5.2) on the activity of lipase Bps-1 were investigated, as shown in Fig. 5A. The enzyme exhibited a favorable tolerance to short-chain alcohols with a large polar index and the enzyme activity presented no significant changes (90%, 110%) after being maintained for

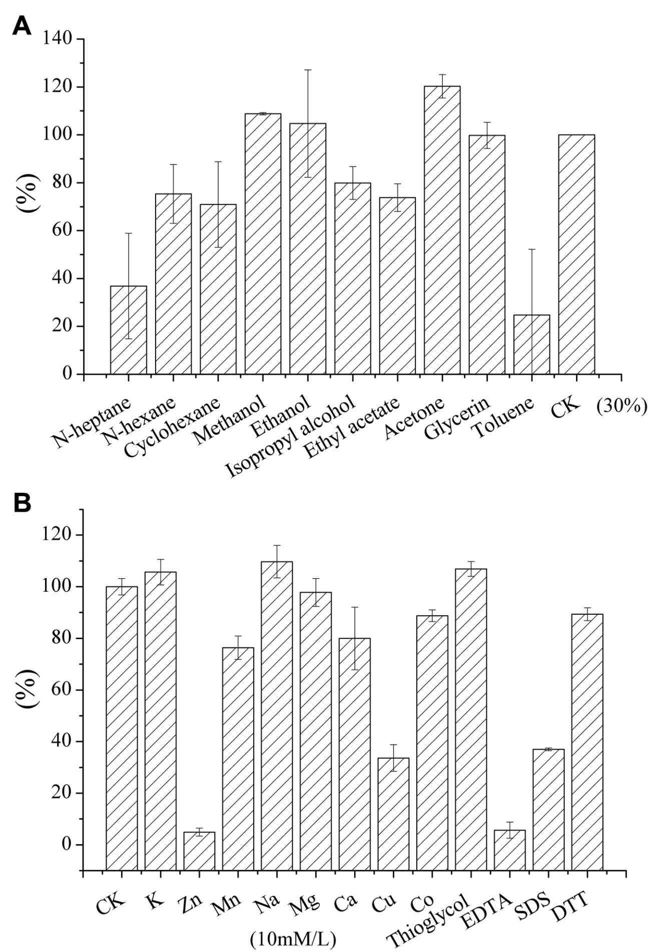

Fig. 5. Effect of organic solvents,metallic ions and protein inhibitors on the activity of lipase Bps-1.

(**A**) The effect of organic solvents on the activity of lipase Bps-1. (**B**) The effects of metallic ions and protein inhibitors on the activity of lipase Bps-1.

1 h in 30% (v/v) methanol and ethanol. However, solvents with low polar indices (methylbenzene, n-heptane, cyclohexane and n-hexane) exhibited an inhibitory effect on Bps-1, whilst acetone and glycerinum exerted an activation effect.

Most lipases show decreasing activity or are inactive in organic solvent environments due to the changes in the secondary structures of the enzyme molecules under the influence of molecules of organic solvents. This triggers a change in enzyme activity in the organic solvents which is mainly shown as conformational changes in the contents of β-sheet and α-helix. Currently, only a small quantity of lipases from *Burkholderia* sp. show levels of tolerance to organic solvents. For example, the lipase from *B. cepacia* S31 retains 43% and 71% of residual activity after separately

being maintained for 6 h in 25% ethanol and acetonitrile [24]. The lipase SL-4 from *Burkholderia ubonensis* maintained residual activity taking up 92~147% of the primary activity after being held for 2 h in 15% (v/v) isopropanol, acetonitrile, acetone, tert-butyl alcohol and chloroform. However, the residual activity remarkably decreased to 28%~70% of the primary activity after holding the lipase in 30% (v/v) xylene solution [22]. The lipases with high tolerance to organic solvents have favorable stability and catalytic activities, and also can influence the enantio-selectivity, thereby showing more significant value for industrial applications.

The Effect of Metallic Ions and Protein Inhibitors on Enzyme Activity

The influences of different metallic ions and inhibitors on lipase activity are illustrated in Fig. 5B. Most previous studies indicate that Ca^{2+} exerted an activation effect on lipases. For example, the lipases from *P. aeruginosa* LX1 [25], *Pseudomonas* DMVR46 [26], *B. cepacia* ATCC25416 [27] and *B. multivorans* V2 [28] all exhibited strong activities due to presence of Ca^{2+}. However, Ca^{2+} had an inhibitory effect on *B. gladioli* Bps-1. The residual activity took up 80% of the primary activity after the Bps-1 was stored in 10 mmol of Ca^{2+} solution for 1 hour. In contrast, K^+ and Na^+ ions were found to enhance enzyme activity. The residual activity accounted for 105% and 110% after the lipase Bps-1 was separately preserved in K^+ and Na^+ solutions for 1 h. This may be because the active site of *B. gladioli* Bps-1 was different from those of the above lipases.

In addition, Zn^{2+} and Cu^{2+} had strong inhibitory effects on Bps-1 lipase: residual activities of Bps-1 lipase only took up 5% and 30% of the initial enzyme activity respectively. The results are in agreement with the reports of Rahman *et al.* [29], showing that transition metal ions at a concentration of 10 mM could enhance the interactions between the ions and the surface amino acids of charged side-chain radicals which significantly influenced the ionization of some amino acid residues, and so lipases became instable due to ion toxicity.

The metal-chelator EDTA (10 mM) had a strong inhibitory effect on *B. gladioli* Bps-1 lipase. Only 10% residual activity remained after the lipase was maintained for 1 h in EDTA, indicating that the lipase was a metallo-enzyme containing disulfide bonds. However, β-mercaptoethanol (10 mM) had no influence on enzyme activity. It is therefore assumed that the disulfide bond in the protein structure of the lipase was not in the active site [30]. Additionally, the inhibitor SDS showed a significant inhibitory effect on the enzyme whilst DTT had no significant effect on activity.

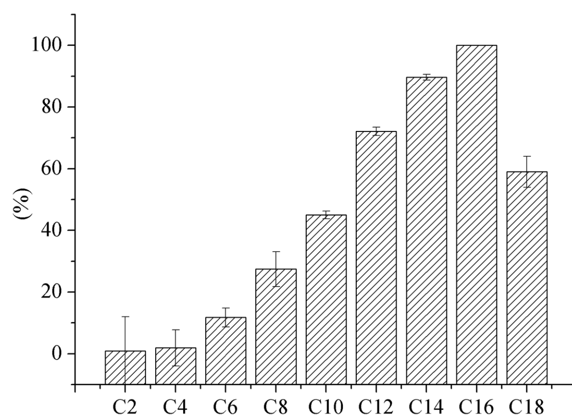

Fig. 6. Substrate specificity of lipase Bps-1.

Substrate Specificity and Kinetic Analysis

The enzyme activities of the lipase were measured separately using C_2, C_8, C_{10}, C_{12}, C_{14}, C_{16}, and C_{18} as substrates to determine the hydrolysis capacity of the lipase on the fatty acid with different lengths of carbon chains. As shown in Fig. 6, the lipase showed differential hydrolysis capacities on the substrates. Enzyme activity was lower during the measurement using C_2, C_8, and C_{10} as substrates whilst higher enzyme activity was observed using C_{12}-C_{18} as substrates. These data imply that Bps-1 exhibited a strong hydrolysis capacity on medium- and long-chain fatty acids. Bps-1 was therefore a true lipase rather than an esterase.

The K_m, V_{max}, and k_{cat} values in the hydrolysis of the substrates C_{12}-C_{18} using Bps-1 were separately measured and summarized in Table 2. Bps-1 exhibited the strongest

Table 2. Kinetic parameters of lipase Bps-1 for p-nitrophenyl esters.

Substrates	V_{max} (μmol min^{-1} mg^{-1})	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1}mM^{-1})
C_{12}	260.60	2.01	158.97	79.09
C_{14}	411.02	1.35	250.72	185.72
C_{16}	480.25	1.05	292.95	279
C_{18}	226.28	1.65	138.03	83.66

hydrolysis capacity on the substrate C_{16}. The enzyme specific activity reached 480.25 U/mg after the substrate C_{16} was hydrolyzed for 10 min in the 50 mM/L Tris-HCl buffer system at a pH of 8.5 at 50°C. Bps-1 had a strong affinity with C_{16} (K_m = 1.05). However, enzyme catalysis was a process during which the reaction rate increased with growing substrate concentration and reached the highest when all active sites of the enzyme were filled with substrates. As the specific activity did not provide an accurate enzyme reaction efficiency model for industrial applications, it was necessary to further calculate k_{cat}/K_m as the catalytic efficiency. As shown in the Table 2, the catalytic efficiency (k_{cat}/K_m) of the lipase Bps-1 gradually rose to the peak from C_{12} (79.09 $s^{-1}mM^{-1}$) to C_{16} (279 $s^{-1}mM^{-1}$) and then reduced to 83.66 $s^{-1}mM^{-1}$ when C_{18} was used as a substrate.

Due to different varieties of enzymes, substrates, and values of K_m, the catalytic efficiency (k_{cat}/K_m) also varies. The value of k_{cat}/K_m is generally between 10^4 to 1 $s^{-1}mM^{-1}$. It was reported by Niu Yu [31] that the lipase TLip produced by *Thauera* sp. had a catalytic efficiency (k_{cat}/K_m) of 71.4 ± 2.4 $mM^{-1}s^{-1}$ when pNPD (C_{12}) was used as the substrate. Li Ai [32] isolated a halophilic lipase (LipS2) produced by *Chromohalobacter canadensis* which also presented better catalytic activity for medium- and long-chain (> C_{12}) triglycerides. When p-NPP was used as the substrate, the values of k_{cat} and K_m were 10.8 mM and 13.2 S^{-1}, and therefore the catalytic efficiency (k_{cat}/K_m) was 73.26 $M^{-1}S^{-1}$. The values of K_m and the catalytic efficiency (k_{cat}/K_m) of the lipase Bps-1 isolated in this research were both at a moderate level, which was probably because *B. gladioli* Bps-1 thrived in suitable conditions without long-term extreme environmental stress; however, it could be modified in future research through genetic cloning and expression as well as mutation. For example, Wang [33] found that the value of k_{cat}/K_m of the lipase RCLpro-RML could be improved to 3833.4 ± 2.6 $s^{-1}mM^{-1}$ after mutation. The increase in this value indicated the enhancement of the catalytic efficiency of the lipase. This suggested that the value of k_{cat}/K_m was of significance for improving the reaction efficiency of the enzyme and reducing the economic cost.

Discussion

At present, a variety of microorganisms capable of producing lipases have been found, and the characteristics of lipases produced by different types of microorganisms differed. The most common strains of productive bacteria include: *Bacillus* sp., *Pseudomonas* sp., *Burkholderia* sp., and *Staphylococcus* sp.; however, the wild strains isolated from the environment have low lipase activity. Bakir [34] isolated 201 strains of thermophilic bacteria from a hot spring in Aydin, Turkey, among which 22 were positive for lipase activity and only one (*Anoxybacillus flavithermus* HBB 134) was found to have a lipase activity of 19.925 U/ml. By using a modified TB-TA plate, we realized the rapid isolation of *Burkholderia* sp. with an isolation rate of greater than 90%. The production of lipases from submerged fermentation of *Burkholderia* sp. Bps1 reached 153.537 U/ml [35]. Shu *et al*. [36] reported that whole cell lipase ZYB002 exhibited good thermal stability and tolerance to organic solvents, and although it's the same as Bps-1 but belonging to the genus *Burkholderia* sp., the optimized lipase activity only reached 22.8 U/ml. The current research revealed that *B. gladioli* Bps-1 has advantages including rapid growth, stable lipase production, and high yield.

The lipase Bps-1, purified through ethanol precipitation, ion-exchange chromatography using HiTrap Q and Superdex 75, had a molecular weight of 34.6 kDa. It exhibited the highest activity in the hydrolysis of p-NPC_{16} at a pH of 8.5 and 65°C. In addition, the values of K_m, k_{cat}, and k_{cat}/K_m were 1.05, 292.95 s^{-1}, and 279 $s^{-1}mM^{-1}$, respectively. After incubating at a pH of 10 for 24 h, the Bps-1 lipase retained 85% of its original enzyme activity. Furthermore, it also had a residual enzyme activity of 50% after incubation at 70°C for 1 h. These indicate that it is a type of alkaline lipase conducive to thermal stability. Lee *et al*. [37] reported that the molecular weight of the lipase purified from *Bacillus thermoleovorans* ID-1 was 34 kDa, and the optimum temperature and pH for the lipase were 70 to 75°C and 7.5, respectively. Fifty percent of its activity was retained after being kept at 60°C for 1 h or at 70°C for 30 min. The enzyme had excellent thermal stability but was incapable of tolerating a strong alkaline environment. Tang *et al*. [38] found that the molecular weight of the lipase produced by *Bacillus coagulans* ZJU318 was 32 kDa and the optimum temperature and pH of the lipase were 45°C and 9.0. The lipase was stable at 7.0 ≤ pH ≤ 10.0 and was rapidly inactivated at temperatures of 40 to 50°C. Ag^+, Cu^{2+}, SDS, Brij 30, and Tween 80 inhibited the activity of the enzyme. Although the lipase from ZJU318 was an alkaline lipase, it had poor thermal stability. Compared with the above two enzymes, Bps-1 has a wider potential range of application.

Ogino [39] believed that the enzymes isolated from microorganisms of high tolerance to organic solvents probably show high tolerance to such solvents. They enriched the microbial samples in a medium containing

20% organic solvent, and finally screened a lipase derived from *Pseudomonas aeruginosa* LST-03 with a certain organic solvent tolerance, however, Shu *et al*. [40] only added a suitable amount of ampicillin and kanamycin to the directional screening medium, and obtained a total of 35 lipase-producing *Burkholderia cepacia* from the four rhizosphere soils. Among them, 15 strains were resistant to 10% by volume of benzene, hexane, and n-heptane. The lipase Bps-1 screened by the aforementioned method also has good short-chain alcohol tolerance and retains 110% of its enzyme activity after storage for 1 h in 30% (v/v) ethanol. This reveals that the lipases produced by *Burkholderia* sp. have excellent stability in organic solvents and therefore can be widely applied in the field of organic synthesis.

In summary, the *B. gladioli* Bps-1 isolated in this research grows rapidly and can yield large quantities of lipase compared with those reported elsewhere. The resulting lipase shows advantages such as high tolerance to alkaline and thermal environments and to short-chain alcohols. Therefore, the lipase can be used as a biocatalyst in the preparation of detergents, in the organic synthetic industry, for transesterification, and so on, and shows particularly high potential for use in the manufacture of biodiesel.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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