Appl Biochem Biotechnol (2010) 162:733–743
DOI 10.1007/s12010-009-8841-3

Purification and Characterization of an Organic Solvent-Tolerant Lipase from *Pseudomonas aeruginosa* CS-2

Ren Peng · Jinping Lin · Dongzhi Wei

Received: 9 July 2009 / Accepted: 26 October 2009 /
Published online: 20 November 2009

Abstract An extracellular lipase secreted by *Pseudomonas aeruginosa* CS-2 was purified to homogeneity about 25.5-fold with an overall yield of 45.5%. The molecular mass of the lipase was estimated to be 33.9 kDa by SDS-PAGE and 36 kDa by gel filtration. The optimum temperature and pH were 50 °C and 8.0. The lipase was found to be stable at pH 4–10 and below 50 °C. Its hydrolytic activity was highest against *p*-nitrophenyl palmitate (*p*-NPP) among *p*-nitrophenyl esters of fatty acids with various chain lengths. The lipase was activated in the presence of Ca^{2+}, while it was inactivated by other metal ions more or less. EDTA significantly reduced the lipase activity, indicating the lipase was a metalloenzyme. Gum Arabic and polyvinyl alcohol 124 enhanced lipase activity but Tween-20, Tween-80, and hexadecyltrimethyl ammonium bromide strongly inhibited the lipase. It exhibited stability in some organic solvents. The lipase was activated in the presence of acetonitrile. Conversely, it was drastically inactivated by methanol and ethanol.

Keywords Organic solvent-tolerant lipase · *Pseudomonas aeruginosa* CS-2

Introduction

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) constitute one of most important groups of industry enzymes, for they are being increasingly exploited for all kinds of fields including detergents, dairy, diagnostics, oil and lipid processing, and biotransformation [1].

R. Peng · J. Lin · D. Wei (✉)
New World Institute of Biotechnology, State Key Laboratory of Bioreactor Engineering,
East China University of Science and Technology, Shanghai 200237, China
e-mail: dzhwei@ecust.edu.cn

R. Peng (✉)
College of Life Science, Jiangxi Normal University, Nanchang 330022, China
e-mail: renpeng23@hotmail.com

Usually lipases catalyze the hydrolysis of water-insoluble triglyceride into di-acylglyceride and mono-acylglyceride, free fatty acid and glycerol, while in immiscible or anhydrous solvents lipases accelerate other chemical reactions such as esterification, transesterification, aminolysis, acidlysis, and alcoholysis.

Generally speaking, use of enzyme in organic media has exhibited many advantages: increased activity and stability; regiospecificity and steroselectivity; higher solubility of substrate and ease of products recovery; ability to shift the reaction equilibrium toward synthetic direction. However, native enzymes almost exhibit low activity in organic solvents—often four or five orders of magnitude lower than in aqueous solutions [2]. Therefore, search for organic solvent-tolerant enzymes has been an extensive area of research [3].

Though enzymes are generally not stable in the presence of organic solvents and are apt to denature, some organic solvent-tolerant lipases have been reported in recent years [4–7]. In a similar effort, we have isolated a *Pseudomonas aeruginosa* CS-2 strain which secretes an organic solvent-tolerant lipase. The present paper describes efficient purification and characterization of the lipase.

Materials and Methods

Materials

DEAE-Sephadex A50 was purchased from Pharmacia. *p*-nitrophenyl palmitate (*p*-NPP) was supplied by Fluka, and protein markers were obtained from Sigma. All other chemicals were obtained from various commercial sources and with highest purity available.

Organism

P. aeruginosa CS-2, an organic solvent-tolerant lipase producer isolated from soil, was used. The accession number of 16 S rDNA of *P. aeruginosa* CS-2 in Genebank was GQ254065.

Cultivation for Lipase Production

The seed culture of *P. aeruginosa* CS-2 was raised at 32 °C (200 rpm) for 12 h in the medium (peptone 0.5%, yeast extract 0.3%, glucose 0.5%, $MgSO_4 \cdot 7H_2O$ 0.05%, NaCl 0.25%, and pH 7.5). The lipase production medium (olive oil 1%, peptone 6%, K_2HPO_4 0.5%, $MgSO_4 \cdot 7H_2O$ 0.1%, gum Arabic 0.04%, and pH 7.5) was inoculated with 5% seed culture and cultivated for 48 h at 37 °C (200 rpm).

Purification of Lipase

The culture supernatant was prepared by removing the cells in culture by centrifugation at 12,000 rpm for 15 min at 4 °C. The supernatant was applied to a hollow fiber ultra-filtration membrane system (membrane pore size, 30 kDa) and the fraction in the hollow fiber ultra-filtration membrane was collected. The resultant solution was passed through another hollow fiber ultra-filtration membrane system (membrane pore size, 50 kDa) and the fraction out of the hollow fiber ultra-filtration membrane was collected. Cold acetone was slowly added to the collected solution to 60% saturation. It was subsequently centrifuged at 12,000 rpm for 20 min. The precipitate was discarded and cold acetone was added again to

the resultant supernatant up to 75% saturation. After centrifuged at 12,000 rpm for 20 min, the precipitate was reconstituted in Tris buffer (20 mM, pH 8.0). The solution was loaded on the DEAE-Sephadex A50 (Ø 2×100 cm). After elution, the fraction with high activity was pooled, and lyophilized to form a powder. It was referred to as purified lipase and was used for further experiments.

Molecular Mass Determination

Molecular mass of the lipase was measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and gel filtration.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed according to the method of Laemmli in a 12% (*w*/*v*) polyacrylamide slab gel [8]. Relative molecular mass was estimated by comparison with molecular mass standards (14.3–97.2 kDa).

Gel filtration was performed with Sephadex G-75 chromatography column (20 mm× 60 cm). Molecular mass standards were: lysozyme 14.3 kDa; α-chymotrypsinogen A from bovine pancreas 25.7 kDa; albumin from chicken egg white 44.3 kDa; and bovine serum albumin 67 kDa.

Protein Assay

The protein concentration was determined by Bradford dye method using bovine serum albumin as a standard [9].

Assay of Lipase Activity

Lipase activity was assayed using *p*-NPP as a substrate. Briefly, the substrate was dissolved in 2-propanol (20 mM). Then the substrate (75 μl) was mixed with 3 ml Tris–HCl buffer (50 mM, pH 8), followed by pre-incubating them at 50 °C for 5 min. The reaction was performed for 10 min at 50 °C by adding 50 μl appropriately diluted lipase to the mixture after pre-incubation for 5 min. The reaction was terminated by adding 1 ml SDS (0.05% *w*/*v*). The absorbance of liberated *p*-nitrophenol was recorded at 410 nm. One unit of lipase activity was defined as the amount of enzyme liberating 1 μmol *p*-nitrophenol in 1 min under standard assay conditions.

Effect of Temperature on Activity of the Lipase from *P. aeruginosa* CS-2 and the Thermal Stability of the Lipase

In order to study the effect of temperature on the lipase activity, the enzyme was determined at different temperatures (20–70 °C). For thermostability tests of the lipase, it was pre-incubated at different temperatures (30–80 °C) for 30 min. Then, the samples were tested for residual lipase activity under standard conditions.

Effect of pH on Activity of the Lipase from *P. aeruginosa* CS-2 and pH Stability of the Lipase

In order to study the effect of pH on the lipase activity, the enzyme was checked at different pH values (4.0–10.0). To determine the effect of pH on lipase stability, the lipase was pre-incubated at 50 °C for 30 min in buffers of pH 3.0–10.0. The residual lipase activity was assayed under standard conditions.

Substrate Specificity

Substrate specificity of the lipase was investigated by using *p*-nitrophenyl fatty acid esters of varying chain length (*p*-nitrophenyl acetate, *p*-nitrophenyl butyrate, *p*-nitrophenyl caprate, *p*-nitrophenyl laurate, *p*-nitrophenyl myristate, *p*-nitrophenyl palmitate, and *p*-nitrophenyl stearate) at 20 mM as substrates and the lipase activity was assayed under standard conditions.

Effect of Various Metal Ions and Surfactants on Activity of the Lipase from *P. aeruginosa* CS-2

The effect of metal ions and surfactants on the lipase activity was investigated by incubating lipase in presence of metal ions (Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, K^{+}, Na^{+}, Fe^{2+}, Zn^{2+}, Sn^{2+}, Co^{2+}, and EDTA) at 1 mM and 10 mM and various surfactants (Gum Arabic, CTAB, Polyvinyl alcohol 124, Tween 20, Tween 80, and Triton X-100). Incubation was performed at 50 °C for 30 min and the samples were tested for lipase activity under standard conditions. The relative activity in presence of metal ions and surfactants was calculated by regarding the control (no metal ions and surfactants in the mixture) as 100%.

Effect of Various Organic Solvents on Activity of the Lipase from *P. aeruginosa* CS-2

Effect of various organic solvents at concentration of 50% (*v*/*v*) on the lipase stability was investigated as follows: 1 ml of organic solvent was added to 1 ml of lipase solution in a sealed glass vial. The mixture was incubated for 3 and 9 h at 30 °C with shaking at 150 rpm. The remaining lipase activity was assayed under standard conditions. The relative activity with organic solvents was calculated by regarding the control (no organic solvents in the mixture) as 100% [10].

Statistical Analysis

Experimental data were presented as mean±SD. Statistical analysis was performed using SPSS 11.5 software.

Results and Discussion

Purification of Lipase from *P. aeruginosa* CS-2

The aim of enzyme purification is to increase specific activity of enzyme while retaining as much as total activity. The extracellular lipase secreted by *P. aeruginosa* CS-2 was purified about 25.5-fold with an overall yield of 45.5%. The purification procedure was summarized in Table 1. In the three-step procedure of purification, DEAE-Sephadex A50 chromatography was the most efficient step, the pattern of which was shown in Fig. 1. Homogeneity of purified lipase was confirmed by SDS-PAGE. Coomassie Brilliant Blue staining revealed a single protein band with a molecular weight of about 33.9 kDa (Fig. 2). Molecular mass of the native lipase was approximately 36 kDa, as determined by size chromatography. Therefore, this suggested the lipase was a monomer. Most of the known *Pseudomonas* sp. lipases have been reported to have molecular mass in the range of 29–33 kDa with a few lipases on higher molecular mass in the range of 45–62 kDa [11]. Therefore, the present lipase was rated between the two ranges of 29–33 kDa and 45–62 kDa. Other three

Table 1 Purification of an extracellular lipase from *P. aeruginosa* CS-2.

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude enzyme	2,270.6	185.2	12.3	100	1
Ultra-filtration[a]	2,025.3	71.7	28.2	89.2	2.3
Acetone precipitation	1,469.1	24.8	59.2	64.7	4.8
DEAE-Sephadex A50	1,033.1	3.3	313.1	45.5	25.5

[a] Ultra-filtration refers to the purification steps using two kinds of hollow fiber ultra-filtration membrane systems (the pore size of the membranes, 30 and 50 kDa)

organic solvent-tolerant lipases produced by *Pseudomonas* sp. strains had molecular masses of about 60 and 27.1 kDa [11–13]. Thus, the molecular mass of *P. aeruginosa* CS-2 lipase agreed closely with that of *P. aeruginosa* LST-03 lipase.

The purification of other organic solvent-tolerant lipases was also reported. Ruchi Gaur et al. purified a lipase from *P. aeruginosa* PseA by ultra-filtration and Sephadex G-100 chromatography with 8.6-fold purification and 51.6% yield [11]. An organic solvent-tolerant S5 lipase was purified by affinity chromatography and anion exchange chromatography [12]. Li Li Zhao et al. succeeded in the purification of an organic solvent-tolerant lipase from *Serratia marcescen* ECU1010, and about 5.5-fold with 15.8% recovery by a five-step procedure was achieved [14].

Effect of Temperature on Activity of the Lipase from *P. aeruginosa* CS-2 and the Thermal Stability of the Lipase

The temperature optimum of the lipase from *P. aeruginosa* CS-2 was observed to be 50 °C, as indicated in Fig. 3. The lipase from *Pseudomonas* S5 had optimal activity at 45 °C and

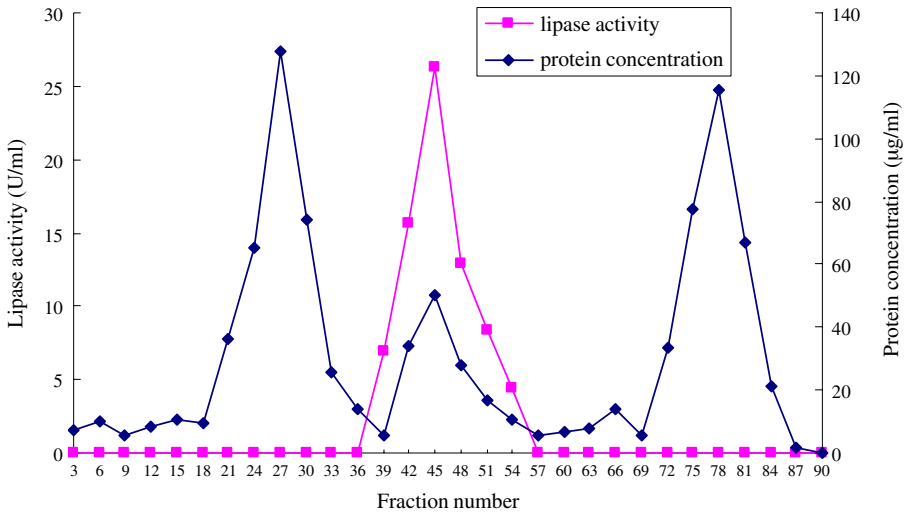

Fig. 1 Elution profile of protein and lipase activity in DEAE-Sephadex A50 column (Ø 2×100 cm) chromatography. The lipase was eluted against Tris–HCl buffer (pH 7, 50 mM) containing NaCl (0.1 and 0.2 mol/L) at a flow rate of 1 ml/min. Each fraction contained 5 ml of eluted solution

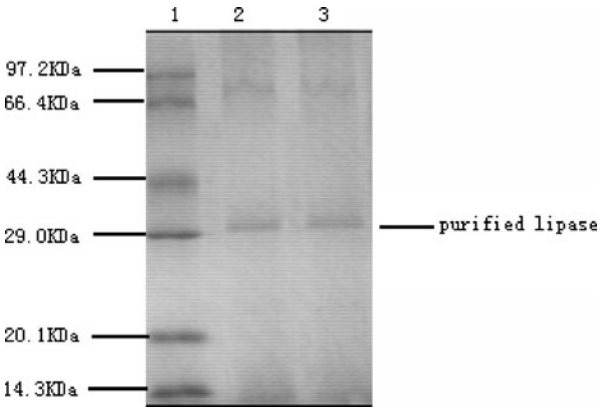

Fig. 2 SDS-PAGE pattern of the lipase from *P. aeruginosa* CS-2. Standard proteins of different molecular (14.3–97.2 kDa; *lane 1*) and the purified lipase (*lanes 2* and *3*) were run in SDS-PAGE (12%) and stained in Coomassie Brilliant blue

LST-03 lipase had maximal activity at 37 °C [12, 13]. The thermal stability profile of the lipase showed that it retained 62.7% of its maximum activity after incubated for 30 min at 70 °C (Fig. 3). This suggested the lipase from *P. aeruginosa* CS-2 showed high thermostability. In contrast, another organic solvent-stable lipase from *P. aeruginosa* LST-03 showed lower stability than *P. aeruginosa* CS-2 lipase [13]. This was in accordance with higher growth temperature of *P. aeruginosa* CS-2 than that of *P. aeruginosa* LST-03 (data not shown).

Effect of pH on Activity of the Lipase from *P. aeruginosa* CS-2 and pH Stability of the Lipase

The optimal pH for the lipase activity was 8.0 (Fig. 4). Similarly, pH optimum of PseA lipase was also found to be 8.0 [11]. The pH stability of the lipase was investigated by

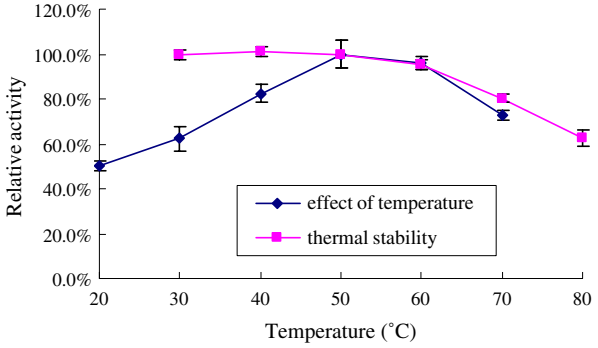

Fig. 3 Effect of temperature on activity of the lipase from *P. aeruginosa* CS-2 and the thermal stability profile of the lipase. Lipase activity was assayed at the temperature indicated, in 50 mM Tris–HCl buffer, pH 8.0, using *p*-nitrophenyl palmitate as a substrate. The optimal lipase activity at 50 °C was taken as 100%. The thermal stability of lipase was investigated by pre-incubating the lipase at different temperature (30, 40, 50, 60, 70, and 80 °C) for 30 min. Residual lipase activity at each temperature was assayed and the lipase activity before pre-incubating the lipase was taken as 100%. All assays were performed in triplicates

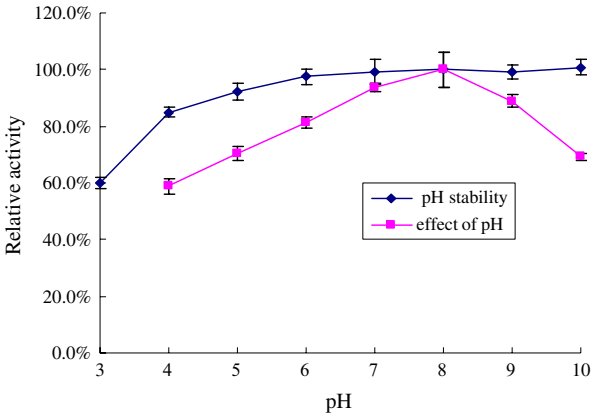

Fig. 4 Effect of pH on activity of the lipase from *P. aeruginosa* CS-2 and pH stability profile of the lipase. Lipase activity was assayed at the pH indicated, in 50 mM Tris–HCl buffer, at 50 °C, using *p*-nitrophenyl palmitate as a substrate. The optimal lipase activity at pH 8 was taken as 100%.The pH stability of lipase was studied by pre-incubating the lipase at different pH (3, 4, 5, 6, 7, 8, 9, and 10) for 30 min. Residual lipase activity at each pH was assayed and the lipase activity before pre-incubating the lipase was taken as 100%. All assays were performed in triplicates

assaying the residual activity of the enzyme after it was incubated at 50 °C for 30 min in buffers of pH 3.0–10.0. *P. aeruginosa* CS-2 lipase displayed stability over a wide range, from pH 4 to pH 10 (Fig. 4). In comparison, LST-03 lipase was very stable at a pH range of 5.0–8.0 for 10 min at 30 °C [13]. The data about pH optimum and pH stability demonstrated that the lipase from *P. aeruginosa* CS-2 was an alkaline lipase.

Substrate Specificity

The activity of lipase from *P. aeruginosa* CS-2 against various substrates was investigated. The results were shown in Fig. 5. The esters of different fatty acids with longer chain were

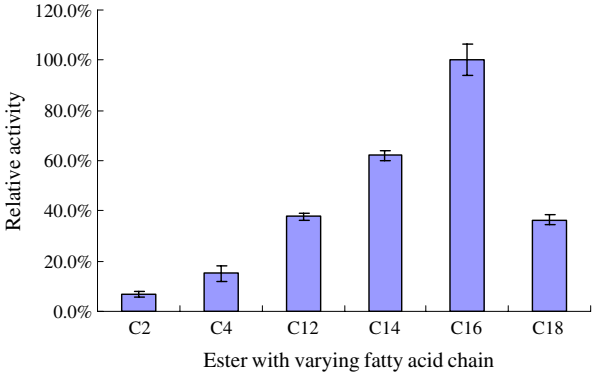

Fig. 5 Substrate specificity of the purified lipase from *P. aeruginosa* CS-2. Lipase assay was performed using *p*-nitrophenyl esters of varying fatty acid chain as substrates. The carbon chain lengths of fatty acid esters used were *p*-nitrophenyl acetate, *p*-nitrophenyl butyrate, *p*-nitrophenyl caprate, *p*-nitrophenyl laurate, *p*-nitrophenyl myristate, *p*-nitrophenyl palmitate, *p*-nitrophenyl stearate (C2, C4, C12, C14, C16, C18). The activity towards *p*-nitrophenyl palmitate was taken as 100%. All assays were performed in triplicates

preferentially catalyzed by the lipase, and *p*-nitrophenyl palmitate was the best substrate as compared to other esters. These were similar to the results of substrate specificity of a lipase from another strain of *P. aeruginosa* [11]. The lipases from *Pseudomonas* S5 and *P. cepacia* also preferentially hydrolyzed substrates with longer chain fatty acids [12, 15]. The substrate specificity of a lipase was usually determined by the size and the hydrophilicity/hydrophobicity of its pockets. Usually, a tunnel-like binding site was more likely to accept substrates with long-chain fatty acids [16]. Therefore, CS-2 lipase presumably had a tunnel-like binding site in its steric structure.

Effect of Various Metal Ions on Activity of the Lipase from *P. aeruginosa* CS-2

The effect of metal ions and metal chelators on the lipase activity was investigated (Table 2) .The results indicated the lipase was found to be stimulated only in the presence of Ca^{2+}, whereas other metal ions inhibited activity of the lipase more or less. The activation and inhibitory effects were also related to the concentration of metal ions. Significant difference in lipase activity was found between treated groups and control ($p<0.01$ or $p<0.05$). Among the tested metal ions, Zn^{2+}, Fe^{2+}, and Mn^{2+} (10 mM) inhibited the activity by 74.7%, 61.3%, and 57.2%, respectively. It was reported most of heavy metal ions inactivated lipase from *Pseudomonas* sp. [11, 12, 17], but Mn^{2+} was found to have a stimulatory effect on the lipase from *Burkholderia multivorans* V2 [18]. The fact that Ca^{2+} activated lipase activity was due to its three distinct roles in lipase action: removal of fatty acids as insoluble Ca^{2+} salts in certain cases; direct enzyme activation resulting from concentration at fat–water interface; stabilizing effect on the enzyme [12]. Furthermore, EDTA significantly decreased the lipase activity, indicating the lipase was a metalloenzyme.

Table 2 Effect of various metal ions on activity of the lipase from *P. aeruginosa* CS-2.

Metal ions	Relative activity (%)	
	Concentration (1 mM)	Concentration (10 mM)
None	100	100
Ca^{2+}	114.7±6.2*	130.5±2.5**
Mg^{2+}	89.0±2.8*	49.7±1.6**
Mn^{2+}	75.6±2.9**	42.8±3.0**
Cu^{2+}	78.0±2.0**	53.7±1.9**
K^{+}	81.4±7.5**	59.8±2.8**
Na^{+}	86.6±4.3*	56.0±1.3**
Fe^{2+}	79.6±2.2**	38.7±3.5**
Zn^{2+}	71.2±2.4**	25.3±1.1**
Sn^{2+}	72.9±3.6**	44.8±3.9 **
Co^{2+}	86.3±4.9*	63.3±5.2**
EDTA	20.2±2.7**	14.4±3.0**

All assays were performed in triplicates

*$p<0.05$; **$p<0.01$, compared with control

Effect of Various Surfactants on Activity of the Lipase from *P. aeruginosa* CS-2

The effect of surfactants on the lipase activity was showed in Table 3. Surfactants reduced the interfacial tension between oil and water and increased the lipid–water interfacial area, and thus they were widely applied to lipase-catalyzed reactions [19]. However, different surfactants had different effects on lipase. For the lipase from *P. aeruginosa* CS-2, there was very significant difference in lipase activity between treated groups and control ($p<0.01$) except the group treated by polyvinyl alcohol 124. TritonX-100, Tween-20, Tween-80, and CTAB inhibited lipase activity, but gum Arabic and polyvinyl alcohol 124 enhanced lipase activity as listed in Table 3. The strong inhibitory effects of Tween-20 and Tween-80 were probably due to their competitive binding to lipase with substrates, while CTAB, a potent cation surfactant, has been thought to destroy the conformation of the lipase. Ruchi Gaur et al. have also reported that CTAB completely inactivated *P. aeruginosa* PseA lipase, but Tween-80 stimulated PseA lipase activity, which is in contradiction to the effect of Tween-80 on activity of CS-2 lipase [11].

Effect of Various Organic Solvents on Activity of the Lipase from *P. aeruginosa* CS-2

The technological utility of enzymes can be enhanced greatly by using them in organic solvents. Consequently, high activity and stability of enzymes in organic solvents are desirable for biotransformation. Effects of organic solvents with various log *P* value at 50% concentration on the lipase activity were studied. The results showed that lipase activity differed significantly between treated groups and control ($p<0.01$ or $p<0.05$). Furthermore, stability of the lipase in organic solvents did not follow log *P* trend (Table 4). Log *P* is an index of the polarity of the solvent and defined as the common logarithm of the partition coefficient of the solvent between *n*-octanol and water. According to log *P* trend, the less hydrophobic the organic solvent, the lower activity of enzyme in the solvent [20]. As indicated in Table 4, the relative activity of the lipase in the presence of methanol and ethanol was less than in the presence of DMSO, whereas log *P* value of DMSO was not higher than that of methanol and ethanol. Furthermore, based on the statistical relationship analysis between relative activity of lipase and log *P* value of organic solvents, *r* value was

Table 3 Effect of various surfactants on activity of the lipase from *P. aeruginosa* CS-2.

Surfactants	Concentration	Relative activity (%)
None		100
Gum Arabic	1.2% (*w*/*v*)	130.0±2.1**
CTAB	1.2% (*w*/*v*)	10.3±1.1**
Polyvinyl alcohol 124	1.2% (*w*/*v*)	109.1±2.6
Tween 20	0.6% (*v*/*v*)	15.5±1.7**
Tween 80	0.6% (*v*/*v*)	14.1±2.3**
Triton X-100	0.6% (*v*/*v*)	59.1±1.5**

All assays were performed in triplicates

**$p<0.01$, compared with control

Table 4 Effect of various organic solvents on activity of the lipase from *P. aeruginosa* CS-2.

Organic solvents	log *P*	Relative activity (%)	
		Incubation for 3 h	Incubation for 9 h
None		100	100
DMSO	−1.3	65.7±1.3**	56.0±2.5**
Methanol	−0.76	10.2±1.7**	6.9±0.8**
Ethanol	−0.24	13.5±1.2**	8.9±0.5**
Acetone	−0.23	45.4±2.3**	22.4±2.4**
Acetonitrile	−0.15	139.8±6.9**	130.6±4.7**
Benzene	2.0	79.2±1.5**	62.2±1.5**
Chloroform	2.0	67.1±3.2**	52.2±5.9**
n-hexane	3.6	86.4±1.9*	69.4±0.7**
Petroleum ether	4.0	79.4±2.8**	72.7±3.9**
Isooctane	4.5	75.0±1.5**	57.4±1.4**

All assays were performed in triplicates

*$p<0.05$; **$p<0.01$, compared with control

only 0.336 for 3 h of incubation and 0.285 for 9 h of incubation, respectively. It was inferred that other factors also influenced the activity of enzyme in organic solvents except log *P* value of organic solvents. The lipase was activated in the presence of acetonitrile, while it was drastically inactivated by methanol and ethanol. It was reported that polar solvents striped off the essential water molecular from the active site of enzymes, and hence activation of the lipase in acetonitrile was puzzling. The paradox could be explained by the hypothesis that acetonitrile increased the access of the substrate to the active site of lipase and it did not destroy the active site. Li Li Zhao et al. reported that the activity of lipase from *Serratia marcescens* retained 1.0% when incubating after 24 h in the presence of DMSO at 50% concentration [14]. The residual activity of LST-03 lipase was 99% when it was incubated for 1 h at 50% DMSO [13]. In this study, the relative activity of 65.7% and 56.0% were kept when CS-2 lipase was incubated for 3 and 9 h at 50% DMSO, respectively. These results indicated that enzyme tolerance to organic solvents differed from lipase to lipase [21]. Owing to the stability of the lipase from *P. aeruginosa* CS-2 in the presence of organic solvents such as acetonitrile, it can be used for organic synthesis and chiral resolution.

Conclusion

An extracellular lipase from *P. aeruginosa* CS-2 was purified to homogeneity. The molecular mass of the lipase was found to be 33.9 kDa by SDS-PAGE and 36 kDa by gel filtration. It exhibited optimum activity at 50 °C and pH 8.0. Among *p*-nitrophenyl esters of fatty acids with various chain lengths, the lipase showed maximum activity on *p*-nitrophenyl palmitate. The lipase was activated by the gum Arabic, polyvinyl alcohol 124, and Ca^{2+} but Tween-20, Tween-80, CTAB, and EDTA had negative effects on its activity. It exhibited the highest level of activity in the presence of acetonitrile among the tested organic solvents.

Acknowledgements The financial support from the Ministry of Science and Technology of the People's Republic of China is thankfully acknowledged (Project ID: 2006AA020203).

References

1. Hasan, F., Shah, A. A., & Hameed, A. (2006). *Enzyme and Microbial Technology, 39*, 235–251.
2. Serdakowsli, A. L. & Dordick, J. S. (2008). *Trends in Biotechnology, 26*, 48–54.
3. Sellek, G. A., & Chaudhuri, J. B. (1999). *Enzyme and Microbial Technology, 25*, 471–482.
4. Ogino, H., Miyamoto, K., & Ishikawa, H. (1994). *Applied and Environmental Microbiology, 60*, 3884–3886.
5. Rahaman, R. N. Z. R. A., Baharum, S. N., Salleh, A. B., & Basri, M. (2006). *Journal of Microbiology, 44*, 583–590.
6. Hun, C. J., Rahaman, R. N. Z. R. A., Salleh, A. B., & Basri, M. (2003). *Biochemical Engineering Journal, 15*, 147–157.
7. Gupta, R., & Khare, S. K. (2006). *Bioresource Technology, 97*, 1788–1793.
8. Laemmli, U. K. (1970). *Nature, 228*, 680–685.
9. Bradford, M. M. (1976). *Analytical Biochemistry, 72*, 248–254.
10. Partridge, J., Halling, P. J., & Moore, B. D. (1998). *Chemical Communications, 7*, 841–842.
11. Gaur, R., Gupta, A., & Khare, S. K. (2008). *Process Biochemistry, 43*, 1040–1046.
12. Rahaman, R. N. Z. R. A., Baharum, S. N., Basri, M., & Salleh, A. B. (2005). *Analytical Biochemistry, 341*, 267–274.
13. Ogino, H., Nakagawa, S., Shinya, K., Fujimura, N., Yasuda, M., & Ishikawa, H. (2000). *Journal of Bioscience and Bioengineering, 89*, 451–457.
14. Zhao, L. L., Xu, J. H., Zhao, J., Pan, J., & Wang, Z. L. (2008). *Process Biochemistry, 43*, 626–633.
15. Pleiss, J., Fischer, M., & Schmid, R. D. (1998). *Chemistry and Physics of Lipids, 93*, 67–80.
16. Krishna, S. H., & Karanth, N. G. (2002). *Catalysis Review, 44*, 499–591.
17. Kojima, Y., & Shimizu, S. (2003). *Journal of Bioscience and Bioengineering, 96*, 219–226.
18. Dandavate, V., Jinjala, J., Keharia, H., & Madamwar, D. (2009). *Bioresource Technology, 100*, 3374–3381.
19. Singh, M., & Banerjee, U. C. (2007). *Tetrahedron Asymmetry, 18*, 2079–2085.
20. Klibanov, A. M. (1986). *Chemtech, 16*, 354–359.
21. Sugihara, A., Ueshima, M., Shimada, Y., Tsunasawa, S., & Tominaga, Y. J. (1992). *Journal of Biochemistry, 112*, 598–603.