Journal of Molecular Catalysis B: Enzymatic 69 (2011) 127–132

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Thermophilic lipase from *Thermomyces lanuginosus*: Gene cloning, expression and characterization

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ARTICLE INFO

Article history:
Received 13 March 2010
Received in revised form 11 January 2011
Accepted 11 January 2011
Available online 18 January 2011

Keywords:
Thermophilic lipase
Water/lipid interface
Biocatalyst
Expression and purification
Esters

ABSTRACT

An extracellular lipase gene *ln1* from thermophilic fungus *Thermomyces lanuginosus* HSAUP$_{03}$80006 was cloned through RT-PCR and RACE amplification. Its coding sequence predicted a 292 residues protein with a 17 amino acids signal peptide. The deduced amino acids showed 78.4% similarity to another lipase *lgy* from *T. lanuginosus* while shared low similarity with other fungi lipases. Higher frequencies hydrophobic amino acids related to lipase thermal stability, such as Ala, Val, Leu and Gly were observed in this lipase (named LN). The sequence, -Gly-His-Ser-Leu-Gly-, known as a lipase-specific consensus sequence of mould, was also found in LN. High level expression for recombinant lipase was achieved in *Pichia pastoris* GS115 under the control of strong *AOX1* promoter. It was purified to homogeneity through only one step DEAE-Sepharose anion exchange chromatography and got activity of 1328 U/ml. The molecular mass of one single band of this lipase was estimated to be 33 kDa by SDS-PAGE. The enzyme was stable at 60 °C and kept 65% enzyme activity after 30 min incubation at 70 °C. It kept half-activity after incubated for 40 min at 80 °C. The optimum pH for enzyme activity was 9.0 and the lipase was stable from pH 8.0 to 12.0. Lipase activity was enhanced by Ca^{2+} and inhibited by Fe^{2+}, Zn^{2+}, K^{+}, and Ag^{+}. The cell-free enzyme hydrolyzed and synthesized esters efficiently, and the synthetic efficiency even reached 81.5%. The physicochemical and catalytic properties of the lipase are extensively investigated for its potential industrial applications.

1. Introduction

Lipase (triacylglycerol acylhydrolases, EC 3.1.1.3), acting only on ester–water interface, can hydrolyze long-chain triglycerides to diacylglycerol and carboxylate, as well as the reverse reaction, synthesis of esters from fatty acids and glycerol [1–4]. In addition, they are also efficient in various reactions such as esterification, transesterification and aminolysis in organic solvents. Apart from their natural substrates, lipases catalyze the chemoselective, the enantioselective and the regioselective hydrolysis and synthesis of a broad range of non-natural esters [5]. So they are of great importance in food industry [6–8], medical applications, biological detergents, textile industry [9–11], and feed industry [12]. Recently, the applications in biodiesel and chiral compounds [13–15] make lipases draw much attention. Therefore, lipases are nowadays extensively studied for their potential industrial applications.

A typical feature of lipases is "interfacial activation", although several examples of lipases have been identified that do not undergo interfacial activation. The active site of a lipase contains a catalytic triad consisting of Ser-His-Asp/Glu. A "lid" or "flap" covers the active site, making it inaccessible to solvent and substrates. The lid opens during process of interfacial activation, allowing lipid substrate access to the active site [14–16].

Lipases are ubiquitous in nature, including plants, animals, and microorganisms. However, only microbial thermostable lipases are commercially significant for their potential use in industries, such as speciality organic syntheses [17–19], hydrolysis of fats and oils, modification of fats, flavor enhancement in food processing [18], resolution of racemic mixtures, and chemical analyses [20–22]. In recent years, there has been increasing interest in thermostable lipases from extreme thermophiles, which would allow enzymatic reaction to be performed at higher temperatures, increase conversion rates, substrate solubility, and reduce contamination of microorganism [23,24]. In addition to thermostability, proteins from thermophiles often show high activity toward organic solvents [25].

Lipases from *Yarrowia lipolytica*, *Aspergillus niger*, *Candida rugosa* and *Rhizomucor miehei* [15,26–28] have been identified, but few from thermophilic fungi. *Thermomyces lanuginosus* is a thermophilic fungi with the ability to grow at high temperatures of 50–60 °C [29]. It is a good candidate in producing thermostable lipases. However, it is often impractical to use them directly due

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doi:10.1016/j.molcatb.2011.01.006

to the low yield of lipase. In this context, a molecular approach through expression of foreign protein in eukaryotic systems has become a good alternative to economically obtain bulk production of lipase. In the present study, we report cloning and sequencing of a new lipase gene from the thermophilic fungus *T. lanuginosus* and its overexpression in *Pichia pastoris*. The effects of thermostability, pH and metal ions on the recombinant enzyme are also shown.

2. Materials and methods

2.1. Strains, plasmids and materials

T. lanuginosus $HSAUP_{03}80006$ was isolated from compost, dung and soil, and preserved by our laboratory [30]. *Escherichia coli* DH5α and plasmid pMD18-T used for gene cloning and sequencing were purchased from Sangon (Shanghai, China). *Pichia* GS115 and expression plasmid vector pPIC9K were obtained from Invitrogen. The media used for culture of *E. coli* and *P. pastoris* GS115 were referred to standardized methods [31].

2.2. Cloning of T. lanuginosus lipase cDNA

T. lanuginosus used in this study was grown in inducing PDA medium containing (g/l): 50.0 g of yeast extract, 20.0 g soluble starch, 18.3 g of olive oil, 5.0 g of K_2HPO_4, 0.15 g of $CaCl_2$, 1.0 g of $MgSO_4{\cdot}7H_2O$ and 15.0 g of agar for 48 h at 50 °C. The total RNA was extracted and first-strand cDNA synthesis was carried out according to Takara RNA PCR Kit 3.0. Two sets of forward and reverse degenerate primers S_1 and A_4 (Table 1) for intermediate fragment of full length cDNA were designed based on the two highly conserved amino acid sequences GHSLG and VPRLP corresponding to ten fungal lipase sequence deposited in GenBank. The 3′ end sequence was obtained with primers S_3A_2 and $M_{13}M_4$ by 3′-RACE while 5′ end sequence was obtained with $S5_1$, $S5_2$, $S5_3$ (Table 1) by 5′-RACE. The full-length cDNA was generated using the sense primers ZS and MX_1.

Phylogenetic tree of this lipase was calculated by ClustalW using the neighbor-joining method. In the analysis, a 1000 bootstrap procedure was used to test the robustness of the nodes on the trees.

2.3. Construction of expression plasmid pPIC9K/ln2

PCR was performed with the plasmid pMD18-T/*ln1* (the full length *ln1* linked to vector pMD18-T) as template using primer Lip-ep5 and reverse primer Lip-ep3 (Table 1), incorporating the *Sna*BI and *Avr*II restriction sites, respectively. PCR product was cloned into vector pMD18-T and transformed into *E. coli* DH5α. Plasmid DNA pMD18-T/*ln* was digested with *Sna*BI and *Avr*II, and then ligated into pPIC9K plasmid. Resulting digested with the same enzymes, pPIC9K/*ln*, was then transformed into *E. coli* DH5α, and sequenced to confirm the correct expression frame.

The plasmid pPIC9K/*ln* was linearized with *Sac*I, resolved on a 1% agarose gel, and purified. 10 μl linearized pPIC9K/*ln* and 80 μl *P. pastoris* competent cells were incubated on ice for 30 min and electroporated into *P. pastoris* competent cells [32] with Eppendorf Electroporator 2510 under 1500 V. All the competent cells were removed to the MD plates after transformation.

2.4. Recombinants screening

After 3–4 days, recombinants grew on MD plates. All the recombinants were transformed onto MM plates. Colonies grew on MD and MM plates were picked up. For further screen, various G418 content (1.0–4.0 mg/ml) was added into YPD plates. PCR detection was performed with 5′a-factor, 3′*AOX1* as primers and plasmids of recombinants as template. Several clones were obtained but only one was chosen for further use and named GS-LN-6.

2.5. Expression and purification of the recombinant lipase

Heterologous expression was under transcriptional control of the *AOX1* promoter in *P. pastoris*. The *P. pastoris* transformants were cultured in 25 ml BMGY medium at 28 °C and 220 rpm. When optical density reached OD_{600} between 2.0 and 4.0, the cells were harvested (8000 × *g* for 5 min), washed and resuspended in 100 ml BMMY medium. The cultures were grown at 28 °C and 220 rpm in 1-l shake flasks for 168 h. The cultures were supplemented daily with 1.0% (v/v) methanol, and sampled every 24 h.

The sample was collected by separating the culture medium from the cells by centrifugation (4 °C, 8000 × *g* for 15 min). After deposited with solid ammonium sulfate reaching 90% saturation for 12 h at 4 °C, the resulting precipitate was collected by centrifugation at 8000 × *g* for 15 min, dissolved in 50 mmol Tris–HCl (pH 8.0) and dialyzed overnight. The dialyzed lipase protein was purified by ion-exchange chromatography on DEAE-Sepharose, equilibrated with Tris–HCl (pH 8.0, 50 mM), according to the manufacturer's instruction (Uppsala, Sweden). The purified recombinant lipase was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [33].

2.6. Lipase activity assays and protein concentration determination

Lipase activity was assayed according to alkali titration method using olive oil as substrate [34,35]. The reaction was carried out in 50 mM pH 9.0 Tris–HCl for 10 min at 60 °C. One unit of lipase activity was defined as the amount of lipase necessary to liberate 1 μmol fatty acid from olive oil per min under the standard assay conditions. Protein content was determined by the method of Bradford [36] with crystalline bovine serum albumin as the standard.

Table 1
The primers for PCR used in the study.

Primer name	Sequence (5′–3′)	Purpose
S_1	5′-GGNCAYAGYTTSGGYGG-3′	First partial fragment
A_4	5′-GGRAGWCTVGGBACNATAT-3′	First partial fragment
S_3A_2	5′-CAGACGGGCGGCACCCTGTAT-3′	3′-RACE
M_{13} Primer M_4	5′-GTTTTCCCAGTCACGAC-3′	3′-RACE
$S5_1$	5′-GTATGGGTGATGCGATACAGGGT-3′	5′-RACE
$S5_2$	5′-TGCAAATGCCCTGTTACCGACGC-3′	5′-RACE
$S5_3$	5′-GCTGCGGCAATAGTTGCCAGT-3′	5′-RACE
ZS	5′-ATGAGGAGCTCCCTTGTGCTG-3′	Full length cDNA
MX_1	5′-GTGCCCATGCATGCAATGCGTT-3′	Full length cDNA
Lip-ep5	5′-TTGCTACGTACGGCCTGTTCGACGAGC-3′	Expression primer
Lip-ep3	5′-GGCCTAGGTTAATCACACTCTGAAATGGG-3′	Expression primer

2.7. Expression stability of the recombinant pPIC9K/ln transformants

Expression stability of the recombinant pPIC9K/*ln* transformant was carried out after conservation on YPD plate for 6 months subculture and ice storage. PCR was applied using Lip-ep5 and Lip-ep3 as primers and cells from the transformant GS-LN-6 as the template. GS-LN-6 was re-expressed and lipase activity was assayed as before.

2.8. Characterization of lipase

To characterize the lipase, each sample contains 200 μg lipase. The 100% activity was obtained with 1 ml sample containing 200 μg lipase under pH 9.0 and 60 °C for 30 min. All the relative activity was calculated as a percentage of this activity.

The optimal temperature was measured after incubation at different temperatures (40–80 °C) for 30 min under pH 9.0. To study the enzyme thermal stability, 1 ml samples either containing 200 μg lipase, were heated to 60, 70, 80 and 90 °C for 10, 20, 30, 40, 50 and 60 min. The residual activity was measured with time. Each measurement was performed three times under pH 9.0.

To attain the optimum reaction pH of the lipase, different pH buffers HCl–KCl (pH 3.0–4.0); H_3COOH–CH_3COONa (pH 4.0–6.0); NaH_2PO_4–Na_2HPO_4 (pH 6.0–7.0); Tris–HCl (pH 7.0–10.0); Na_2HPO_4–NaOH (pH 10.0–13.0) were used. The effect on enzyme stability was employed by measuring the remaining activity after incubation for 30 min at 60 °C in different pH buffers.

Al^{3+}, Fe^{2+}, Zn^{2+}, K^+, Ag^+, Li^+, Mg^{2+}, Na^+, Ba^{2+} and Ca^{2+} (final concentration 50 mmol/L) were used to determine effects of metal ions on lipase activity. The enzyme was incubated with each metal ion and then the residual activity was measured at 60 °C and pH 9.0.

2.9. Lipase-catalyzed biodiesel synthesis reaction in solvent-free system

Different molar ratios of methanol and oleic acid were analyzed. After 1.5 ml methanol, 3.9 ml oleic acid (molar ratio 1/1) and 4.6 ml 50 mM glycin–NaOH buffer (pH 9.0) were mixed in a 50 ml capped glass vial and incubated at 45 °C for 10 min, 1 ml rude enzyme solution was added and shook at 150 rpm for 24 h at 45 °C in constant temperature electric water bath. Further reactions were carried out with various substrate molar ratios and silica gel (5 g) [37].

3. Results and discussion

3.1. Cloning of T. lanuginosus lipase gene ln1

The full length cDNA was obtained from three fragments. First 191 bp fragment product was obtained using degenerated primers. The partial sequence was 75.5% identical to the lipase gene *lgy* of *T. lanuginosus*. The 3′ end and 5′ end sequence were extended by RACE-PCR. A 1095 bp full-length sequence of *T. lanuginosus* was obtained by PCR using primers ZS and MX_1. The cDNA (GenBank accession number EU004197) sequence analysis resulted in an open reading frame of 868 bp. It also contained high conservation in -Gly-His-Ser-Leu-Gly-, which was known as a lipase-specific consensus sequence. SignalP3.0 Server program analysis revealed it contained a putative signal peptide of 17 amino acids which was identical to the registered lipase gene (AF054513) of *T. lanuginosus*.

Amino acid sequence was used to search the National Center for Biotechnology Information (NCBI) database to identify the protein by the Protein-Protein Basic Local Alignment Search Tool program (BLASTP). It revealed this lipase belonged to the Lipase 3 family of Lipase Super family. So we can conclude we obtained a lipase gene.

Amino acid residues forming the catalytic triad in fungi lipases are completely conserved, and the serine residue, one of the catalytic triad, is also located in a highly conserved pentapeptide motif. Combination with other fungi lipase genes we conclude that the catalytic triad of LN is formed by Ser168, Asp204, and His280. Based on the gene sequences from other lipases, phylogenetic tree was constructed (Fig. 1). Analysis indicated that our lipase gene shared the highest similarity of 78.4% with lipase from *T. lanuginosus* (EU022703).

3.2. Expression and purification of the recombinant lipase

For further characterization of *T. lanuginosus* lipase, plasmid pPIC9K/*ln* was constructed and electroporated into *P. pastoris* cells. By screen, transformant strain GS-LN-6 was chosen. After induction with 1.0% methanol, recombinant lipase was secreted into culture medium in an active form under transcriptional control of the *AOX1* promoter in *P. pastoris*. The induced crude extract containing the lipase were analyzed by SDS-PAGE (Fig. 2a). After purified by ion-exchange chromatography on DEAE-Sepharose, a band (about 33 kDa) corresponding to the lipase was isolated from induced crude supernatant (Fig. 2b).

The relative molecular mass of the recombinant lipase is higher than deduced 31.4 kDa amino acid sequence. This suggests that post-translational modifications exist in the recombinant lipase. Bioinformatics analysis also showed that there were two potential N-glycosylation sites. Moreover, the purified recombinant lipases gave a positive periodic acid-Schiff staining reaction, which shows that the recombinant lipases were modified by glycosylation (data not shown). These data indicated the molecular mass change is caused by glycosylation. A more in-depth investigation is required

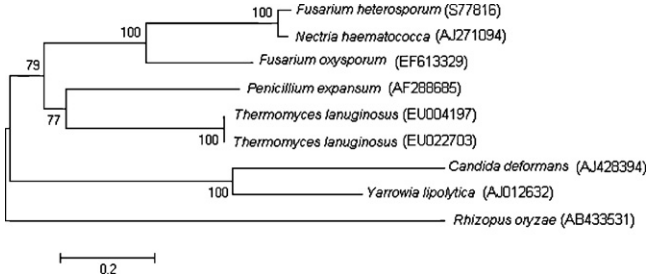

Fig. 1. Phylogenetic tree of the lipase genes. The tree was generated by gene sequence alignment with ClustalW using the neighbor-joining method. Bootstrap support values over 70 are shown.

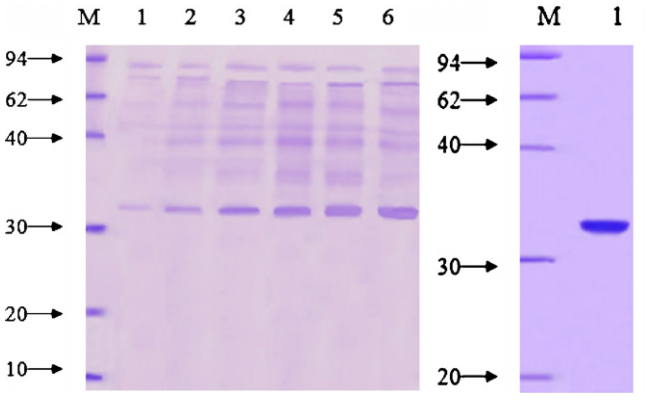

Fig. 2. (a) SDS-PAGE analysis of the recombinant expression lipase from *P. pastoris*. Lane M, protein molecular weight marker (94, 62, 40, 30, 20 and 10 kDa from the top); lanes 1–6, 1, 2, 3, 4, 5 and 6 days, respectively. (b) SDS-PAGE analysis of purified expression lipase LN. Lane M, protein molecular weight marker (94, 62, 40, 30 and 20 kDa from the top); lane 1, purified recombinant lipase.

to explore the effect of glycosylation modification. It is also presumed that the site difference, extent difference, conformation difference of glycosylation modification, and the obstacle of oligosugar branch to the substrate combination, etc. could influence the specific activity of lipase [27].

3.3. Lipase activity assays and protein expression determination

After 144 h induction expression of transformant GS-LN-6, lipase hydrolysis activity of the culture supernatant reached 61 U/ml. By only one step purification on DEAE-Sepharose column, activity increased to 1328 U/ml. This expression level of the recombinant lipase in *P. pastoris* GS115 was higher than that of other fungi lipase genes [15,39,40].

3.4. Expression stability of the recombinant lipase

No obvious genetic and expression activity variation was observed after subculture for 30 times. It was still easy to get the 825 bp coding sequence by PCR with primers Lip-ep5 and Lip-ep3 from transformant GS-LN-6. And the specific activity of induced recombinant lipase remained 57 U/ml. It indicates that exogenous *ln* can be inherited with yeast genome steadily in *P. pastoris* GS115. From this data, we can conclude that transformant GS-LN-6 is stable in studies of protein engineering to improve its properties and then to further extend the scope of its industrial application.

3.5. Thermostability

Fig. 3a shows the optimum reaction temperature was 60 °C with 100% activity as 2.63 U/μg, and the enzyme was stable at temperatures of 40–70 °C. As shown in Fig. 3b, activity of the enzyme changed little at 60 °C (pH 9.0) after 1 h. It kept even 55%, 45% and 40% activity after 60 min incubation at 70 °C, 80 °C and 90 °C respectively. It was proved to be a thermophilic lipase in fungi lipases [41].

Thermostable enzymes have long been of interest to biochemists. Our recombinant lipase LN exhibited remarkable thermal stability for activity, but the lipase from *Rhizopus oryzae* lost activity completely at 50 °C after 30 min incubation. The recombinant lipase from *C. rugosa* suffered inactivation when heated to 50 °C. A lipase of *Geotrichum candidum* was stable up to 50 °C and lipase from *Penicillium expansum* showed no residual activity after 15 min at 50 °C [38–40,42]. Taken together, it can be seen that almost all reported lipases from fungi were inactivated at 50 °C except for lipases from *T. lanuginosus*. Our result showed that the *T. lanuginosus* lipase was one of the most thermostable enzymes among the published lipases in eukaryotic organisms. The high temperature stability of LN may make it useful in applications. Therefore, this highly stable lipase is more suitable for using in cosmetics. It has been suggested that the enzymes of thermophilic fungi are appreciably thermostable, but less so than those of hyperthermophilic archaea. Since the major enzymatic action occurs at 40–60 °C in most operational situations, thermostable enzymes from thermophilic fungi may be better suited than enzymes from hyperthermophiles [43]. Its tolerance to high temperature will make it advantageous in industry.

Mechanisms for thermostability of thermophilic proteins have been studied [44]. Protein hydrophobicity has been shown to be one of the mechanisms. Hydrophobic amino acids make proteins have great hydrophobicity, which increases protein thermostability [45]. It was observed that Ala frequently appears in the core of thermophilic proteins [46]. It is thought that Ala at the protein core is concerned with compact packing, which would stabilize the core of proteins [47,48]. In our study, higher frequencies Ala, Val, Leu and Gly were also observed in LN. Further characterization of these amino acid residues is necessary for comprehensive understanding

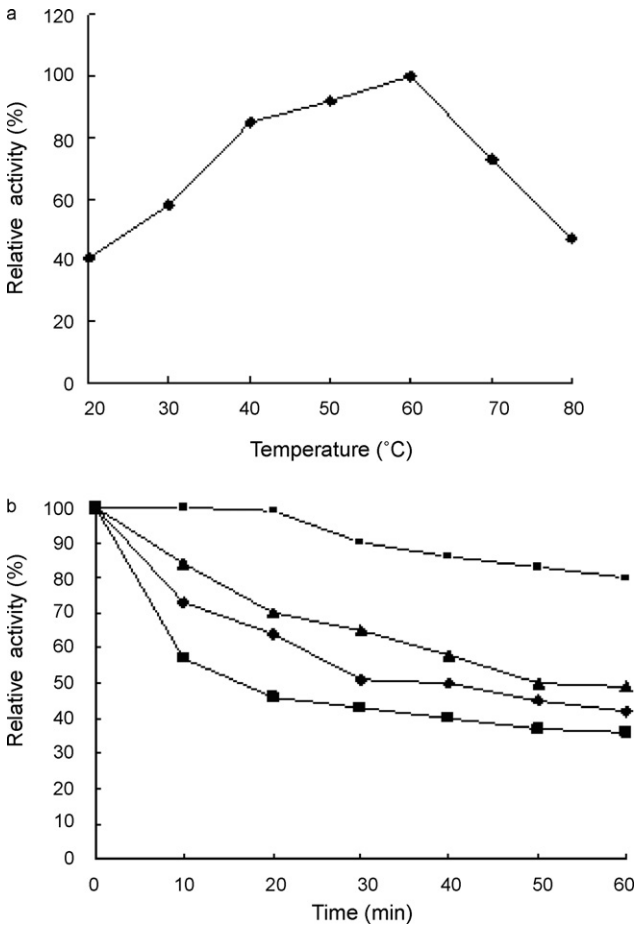

Fig. 3. (a) The optimum reaction temperature of expression lipase. (b) The thermostability of expression lipase. The relative activity at 60 °C (■), 70 °C (▲), 80 °C (♦), and 90 °C (■) for 10, 20, 30, 40, 50 and 60 min were monitored throughout the experiment, and were calculated by taking the activity of pH 9.0 and 60 °C as 100.

of their role in thermostability lipase LN. It is also proposed that glycosylation of lipase helps to improve thermostability. The possible explanation is that in many enzymes, glycosylation is involved in protein rigid structure formation which increases the thermostability [6].

3.6. Effects of pH on lipase activity

The effect of pH on enzyme activity and stability is shown in Fig. 4a and b, and 100% activity was obtained as 2.63 U/μg. The optimal pH for the lipase activity was approximately 9.0, and LN was able to retain 95% of the full activity when incubated in the buffer pH 9.0 for 1 h. The enzyme was relatively stable over a broad pH range, from pH 8.0 to 12.0 as shown in Fig. 4b.

Interactions occurring between substrate and active site in the process of interfacial catalysis are highly dependent on classical parameters such as pH [49]. The effects of pH on enzyme turnover have mostly been studied in connection with structural features of the active site. For example, the optimum activity of gastric lipase recorded at acidic pH values clearly illustrates the effects of pH on the enzyme adsorption rates [49]. It acts optimally at acidic pH because it binds to the interface preferentially at low pH values. For a long time, almost lipases reported showed inactivated up to pH 8.5. The *R. oryzae* lipase activity decreased rapidly at pH above 8.5 [50]. The optimum pH of *C. rugosa lipl* was at 7.0 and decreased to 60% at pH 8.0 [39]. *R. oryzae* lipase in *P. pastoris* was measured at pH 8.1 and outside this range its activity lost rapidly [42]. In this study,

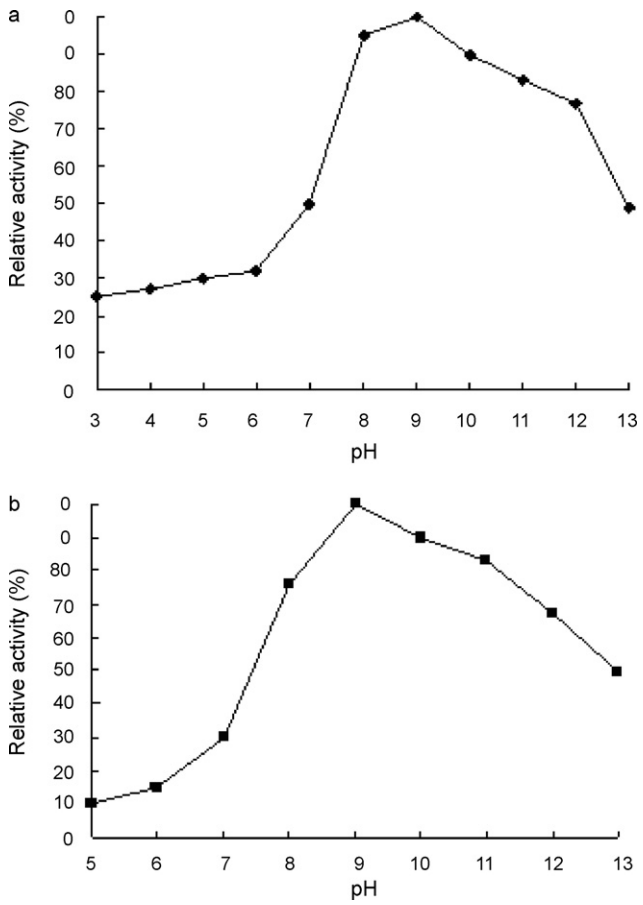

Fig. 4. (a) The optimum reaction pH of expression lipase. (b) The pH stability of expression lipase.

we cloned a lipase present optimal activity at alkali pH 9.0 and stable from pH 8.0 to 12.0. Our results indicated the *T. lanuginosus* lipase was one of the few enzymes that can tolerant to high alkaline request among the published lipases from eukaryotic organisms. It makes this *T. lanuginosus* lipase exit enormous potential capacity in biological detergent industry.

3.7. Effects of metal ions and chemical reagents on lipase activity

The lipase activity was found to be enhanced by Ca^{2+} up to 141%, whereas Li^{+}, Mg^{2+}, Na^{+}, and Ba^{2+} showed no effect. K^{+} and Zn^{2+} inhibited the enzyme activity by 57% and 66% respectively. Ag^{+} and Al^{3+} inactivated the enzyme less than K^{+} and Zn^{2+}, but more than Fe^{2+} (Table 2).

3.8. Lipase-catalyzed biodiesel synthesis reaction in solvent-free system

To quantify the alkyl ester synthesized in solvent-free medium, initial and residual oleic acid were titrated with 0.05 mol/l NaOH. From Table 3 we could know 50.4%, 78.9%, 66.7%, 55.8%, and 43.

Table 2
Effects of different metallic ions on expression lipase LN activity.

Metal ion	Relative activity (%)	Metal ion	Relative activity (%)
K^{+}	43	Fe^{2+}	87.1
Ba^{2+}	104	Na^{+}	97.9
Mg^{2+}	99.5	Al^{3+}	69.9
Ag^{+}	57.9	H_2O	100
Zn^{2+}	33.6	Ca^{2+}	141

Table 3
Utilization of the initial free fatty acids (oleic acid).

Oleic acid (ml)	Methanol (ml)	Molar ratio	Conversion (%) without silica gel	Conversion (%) with silica gel
1	3.9	2:1	50.4	54.7
0.5	3.9	1:1	78.9	81.5
0.25	3.9	1:2	66.7	61.0
0.17	3.9	1:3	55.8	50.5
0.125	3.9	1:4	43. 6	49.5

6% of the initial free fatty acids (oleic acid) were converted to alkyl esters when silica gel was not added into the mixture. With silica gel 54.7%, 81.5%, 61.0%, 50.5% and 49.5% of the initial free fatty acids (oleic acid) were converted after 24 h, respectively. The alkyl ester synthesized and oleic acid consumed reached a maximum value in 24 h and did not increase with the time.

We utilized different molar ratio to generate the largest ester formation with or without silica gel. Ultimately, we got the maximum esters conversion 81.5%, when acid/methanol molar ratio was 1:1. This was more than the 61.0%, 50.5% and 49.5% when molar ratio was 1:2, 1:3 and 1:4 with silica gel even. Actually, most experiments showed that the optimal esters yield was obtained with acid/methanol molar ratio 1:1. In contrary, when acid excess (acid/methanol molar ratio 2:1) or methanol excess (acid/methanol molar ratio 1:4), synthesis rate dropped, and the product mixture contained large quantities of residual methanol, and sometimes mono- and diglycerides [44]. This may be a result of the inhibition of increasing acid and methanol on enzyme. We also see from Table 3 that silica gel make the conversion rate increase. The increasing conversion rate might be caused by absorption of silica gel on the generated water of reversible reaction. But even the starting reaction composition was different, all the reactions ended within 24 h. That was to say the reversible synthesis and decomposition reached equilibrium, and the ester generation reached maximum in 24 h.

4. Conclusion

This study reported the cloning, expression and characterization of lipase LN. The gene *ln* was obtained in a simple and rapid way by PCR and then inherited with the yeast genome stably. The biologically active form of this lipase was successfully over expressed in *P. pastoris*. Recombinant enzyme was purified by only one step DEAE-Sepharose anion exchange chromatography and showed high thermo stability up to 90 °C for 15 min. The expressed lipase activity was relatively stable from pH 8.0 to 12.0 and showed maximum activity at pH 9.0. Taken together, for the first time, we cloned a gene encoding the lipase have both high thermostability and high alkali tolerance. Meanwhile, the enzyme could hydrolyze and synthesize esters efficiently. Therefore, the enzyme has potential of broad applications in alkyl ester synthesis, biofuels production and especially biological detergent via cell-free synthetic enzymatic pathway biotransformation in cosmetics.

Acknowledgements

We thank Dr Anna Li for her careful revision and technical help on the manuscript. This work was supported by the Chinese National Nature Science Foundation and Chinese National Programs for High Technology Research and Development.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2011.01.006.

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