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CLONING AND SEQUENCE ANALYSIS OF COMPLETE GENE ENCODING AN ALKALINE LIPASE FROM *Penicillium cyclopium*

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Recieved

The complete gene (PG37 *lip*I) encoding an alkaline lipase (PG37 LipI) was cloned from the genomic DNA of *Penicillium cyclopium* PG37. The cloned PG37 *lip*I is 2020 bp in length, consisting of 632 bp of the 5' flanking promoter region and 1388 bp of the downstream fragment that contains 6 exons and 5 short introns. The promoter region harbors putative TATA box, CAAT box and several transcription factor binding sites. The open reading frame (ORF) encodes a PG37 LipI of 285 amino acid residues, which was predicted to contain a 20-aa signal peptide, a 7-aa propeptide and a 258-aa mature peptide with a conserved motif Gly-X-Ser-X-Gly. However, PG37 LipI shows only 32%, 30%, 28% and 26% identity with lipases of *Aspergillus parasiticus, Penicillium camembertii*, *Thermomyces lanuginosus* and *Rhizomucor miehei*, respectively. It was predicted that the main secondary structures of PG37 LipI are α-helix and random coil. Three amino acid residues, Ser^{132}-Asp^{188}-His^{241}, compose the enzymatic active center in the tertiary structure.

Lipases (triacylglcerol acylhydrolases, EC 3.1.1.3), which exist ubiquitously in living organisms, can catalyze the hydrolysis of triacylglycerols into diacylglycerols, monoacylglycerols, free fatty acids and glycerol at the oil-water interface, as well as the synthesis of lipids, esterification, transesterification and aminolysis reaction in organic solvents [1, 2]. Recently, the enzymes have been widely applied in food, detergent, pharmaceutical and cosmetic industries, and also used as biosensors, diagnostic tools and in bioremediation, etc [3]. During the past decades, lipases have been produced and purified from mammalian, bacterial, fungal and plant sources [4, 5]. Their physicochemical properties such as molecular weight, amino acid composition, metal ion-binding capacity and substrate specificity have also been studied in detail. A number of lipase genes have been cloned from yeast, *Bacillus subtilis*, and *Rhizopus* species [6–8]. Although there are wide differences in their primary structures and characteristics, most of the lipases share the same core topology in the tertiary structure, known as the α/β hydrolase fold [9]. All lipases studied so far share a conserved motif Gly-X-Ser-X-Gly in the primary structure, where the serine residue is considered as the catalytic residue that is one of catalytic triad Ser-Asp-His, and the 2 flanking glycine residues are essential for topology [10]. The catalytic triad locates in the hydrophobic cleft of the lipase, where the fatty acyl chain inserts and gets cleaved.

Until now, it has been found that over 60 genera of microorganisms can produce lipases [11]. In contrast to animal and plant lipases, extracellular microbial lipases can be produced by fermentation in large quantities and relatively inexpensively [12]. But further commercialization of the microbial lipases is hindered by their low stability, activity or specificity. In order to make the use of lipases more economical and efficient, more attention has been focused on modifying their structures and improving their catalytic properties with chemical or physical approaches and, recently, through genetic engineering means [1]. Recombinant DNA and genetic engineering technology not only provided the most powerful tools for deciphering the relationship between the structure and function of lipases, but also made it possible for their rational modification and higher production. Niu et al. [13]. utilized error-prone PCR to introduce mutations in the *Rhizopus arrhizus* lipase. Through screening, one of the mutant lipases, A9T-E190V-M225I with improved thermostability was obtained. The optimum temperature of the mutant lipase was 10°C higher that of the wild-type. In addition, the half-life of the mutant lipase at 50°C exceeded that of wild-type by 12-fold. Cox et al. [14] successfully increased the *Pseudomonas alcaligenes* lipase production by 15% through introducing a mutation, C-14T, in the promoter region of *P. alcaligenes* lipase gene.

In previous studies, we isolated the strain of *Penicillium cyclopium* PG37 that is able to secrete an alkaline lipase with high activity. Recently, the cDNA sequence encoding PG37 LipI was cloned based on the determined N-terminal amino acid sequence (GenBank accession AF274320) [15]. However, knowledge of the transcriptional regulation of PG37 *lip*I is still unclear. The aim of this work was cloning of the complete DNA gene *lip*I encoding PG37 LipI from the genomic DNA of *P. cyclopi-*

um PG37 by two steps of PCR amplification, usual PCR and ligation-mediated PCR. Furthermore, the DNA sequence of PG37 *lip*I and its deduced amino acid sequence were analyzed by bioinformatics. To our knowledge, this is the first report on the cloning and sequence analysis of a complete lipase gene from *P. cyclopium*.

MATERIALS AND METHODS

Strains, plasmids, and media. *Penicillium cyclopium* PG37 strain, isolated from soil in China as reported previously [15], was used for the alkaline lipase production, as well as for genomic DNA preparation. The following liquid medium was used for *P. cyclopium* PG37 strain culture (g/l): soybean phospholipids – 7.5, of corn steep liquor – 30, soybean meal hydrolyzate – 300 ml, K_2HPO_4 – 10.0, $MgSO_4$ – 1.0, trisodium citrate (pH 7.5) – 0.5. *Escherichia coli* JM109 strain was used as host cell for gene cloning and DNA sequencing, and it was cultured on Luria-Bertani medium [16]. pUCm-T vector for cloning of PCR products was purchased from Sangon (China).

Reagents. Restriction enzymes used for gene cloning, X-gal, IPTG, T4 DNA ligase, *Taq* DNA polymerase and DNA marker were purchased from TaKaRa (China). EZ-10 Spin Column DNA Gel Extraction Kit and EZ-10 Spin Column Plasmid Mini-Preps Kit were purchased from BBI (Canada). All other chemicals were of analytical grade.

Primers for PCR amplification. With the information of the cDNA sequence encoding PG37 LipI (GenBank accession AF274320), PCR primers F1 (5'-ATGTTGT-TCAACTACCAATC-3'), R1 (5'-CAAATCAGATG-TATTTTTAT-3'), R2 (5'-CCTCGACTCCAAAATAG-GTG-3') and R3 (5'-GATTGGTAGTTGAACAA CAT-3') were designed. Based on the principle of the ligation-mediated PCR, the oligonucleotides LD (5'-ATCCCTTCACTCTCAAGTGG-3') and 8-N (5'-AATTC CAC-3') were designed and used as an adaptor to be ligated to the genomic DNA digested with restriction enzyme *Eco*R. F1 and R1 were used for PCR amplification of the downstream fragment of PG37 *lip*I, while LD and R2 or R3 were used for PCR amplification of the promoter region (upstream fragment of PG37 *lip*I). All primers were synthesized by Sangon (China).

Genomic DNA extraction. Extraction of genomic DNA from *P. cyclopium* PG37 was performed according to the method [17] with appropriate modification. *P. cyclopium* PG37 was cultivated on above mentioned liquid medium at 28°C for 24 h on a rotary shaker at 220 rpm. The mycelia were collected through filtration and washed three times with SE buffer (0.15 mM NaCl, 0.1 M EDTA, pH 8.0). The washed mycelia were homogenized in 10 volumes (w/v) of DNA extraction buffer (0.2 M Tris-HCl, 0.5 M NaCl, 10 mM EDTA, 2% SDS, pH 8.0) for 3–5 min, and then incubated at 65°C for 1 h with shaking at 100 rpm. The homogenate was cooled at room temperature and mixed with isovolumic phenol/chloroform/isoamylalcohol (25 : 24 : 1), followed by vigorous vortexing for 15 s. The supernatant was collected by centrifugation at 15000 rpm at 4°C for 10 min and re-extracted with isovolumic chloroform/isoamylalcohol (24 :1). 2.5 volumes (v/v) of ethanol were added to the resulting supernatant and then allowed to stand at –20°C for 30 min, followed by centrifugation at 15000 g for 10 min. After washed twice with 75% ethanol and air dried, the resulting DNA precipitate was dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) supplemented with 50 μg/ml RNase.

Cloning of the downstream fragment. The downstream fragment of PG37 *lip*I was amplified from genomic DNA of *P. cyclopium* PG37 using primers F1 and R1, which were located at the 5'- and 3'-ends of the cDNA sequence. The aimed band was purified with DNA Gel Extraction Kit and ligated to pUCm-T vector. The resulting mixture was transformed into *E. coli* JM109 competent cells, followed by blue-white screening. The recombinant plasmid was identified by PCR amplification.

Cloning of the promoter region. Ligation-mediated PCR amplification of the promoter region was performed by four steps, as shown in Fig. 1. The genomic DNA of *P. cyclopium* PG37 was digested with restriction enzyme *Eco*RI. The derived DNA was ligated with an adapter, which was an equimolar mixture of two oligonucleotides, 8-N and LD. The ligated DNA sample was amplified with primers LD and R2, and then subjected to a second round of PCR amplification with primers LD and R3 for confirmation. The purified PCR product was ligated to pUCm-T vector for sequencing.

DNA sequence analysis. By aligning the nucleotide sequences of the 2 cloned DNA fragments, the complete gene encoding PG37 LipI was obtained. The sequence of PG37 *lip*I was analyzed by bioinformatics. The prediction of promoter region and its characterization was carried out using the TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html), along with the PLACE (http://www.dna.affrc.go.jp /PLACE/signalscan.html). For the exact localization of the exon/intron boundaries, the GeneMark (http://opal.biology.gatech.edu/GeneMark/eukhmm.cgi) was used. The ORF was determined by the program of NCBI ORF Finder (http://www.ncbi.nlm.nih. gov/gorf/gorf.html).

Amino acid sequence analysis. The deduced amino acid sequence of PG37 LipI was analyzed by the Expert Protein Analysis System (http://www.expasy.org/). The ProtScale (http://au.expasy.org/tools/protscale.html) and the TMHMM (http://www.cbs. dtu.dk/services/TMHMM-2.0/) were used for predicting the hydrophobic regions and the transmembrane domains, respectively. The SignalP 3.0 (http://www.cbs.dtu.dk/ services/SignalP/) was used to identify the signal peptide of PG37 LipI. The physicochemical properties of PG37 LipI were analyzed by Protparam (http://au.expasy. org/tools/protparam.html). Motifs of PG37 LipI were

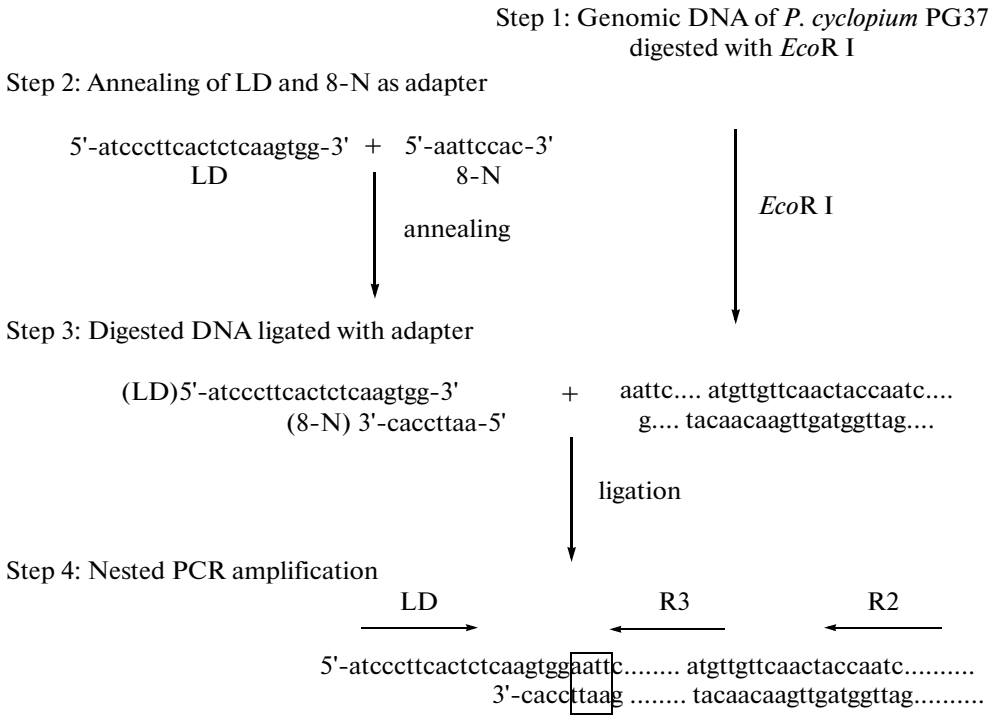

Fig. 1. Diagramatic representation of the ligation-mediated PCR amplification.

predicted by the InterProScan (http://www.ebi.ac.uk/Tools/InterProScan/). Protfun (http://www.cbs.dtu.dk/services/ProtFun/) was used for the function prediction. Secondary and tertiary structures were predicted by SOPMA (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) and ESyPred3D (http://www.fundp.ac.be/sciences/biologie/urbm/bioinfo/esypred/), respectively.

RESULTS AND DISSCUSSION

Cloning of the downstream fragment of PG37 *lipI*. According to the cDNA sequence of PG37 LipI, a forward primer F1 located at ATG start codon region and a reverse primer R1 complementary to transcription terminal region were used to amplify the downstream fragment of PG37 *lip*I from the genomic DNA of *P. cyclopium* PG37. A 1.3 kb DNA fragment was amplified (Fig. 2a, lane 1), and then purified and cloned for sequencing. Result showed that the cloned downstream fragment of PG37 *lip*I is 1.316 bp in length and contains the complete nucleotide sequence of the cDNA with 5 short intervening introns ranging from 50 to 70 bp as indicated in Fig. 3.

Cloning of the promoter region of PG37 *lipI*. Amplification of the unknown 5' flanking promoter region of PG37 *lip*l was based on a ligation-mediated PCR approach [15]. The extracted genomic DNA of *P. cyclopium* PG37 and the digested DNA to which an adapter was ligated were indicated in Fig. 2b. Using the ligated DNA sample as template, a 750 bp DNA fragment was amplified with primers LD and R2 (Fig. 2a, lane 2) and then cloned on pUCm-T vector. To verify the result, the recombinant vector was subjected to a second round of PCR amplification with primers LD and R3 (Fig. 2a, lane 3), followed by sequencing. Result showed that the cloned promoter region of PG37 *lip*I is 740 bp in length, harboring putative TATA box, CAAT box and several transcription factor binding sites.

DNA sequence characterization of PG37 *lipI*. There was 36 bp in common between 3'-end of 740 bp promoter region and 5'-end of 1.316 bp downstream fragment. By aligning, the complete gene PG37 *lip*I is 2.020 bp in length as shown in Fig. 3. Compared with cDNA sequence, 632 bp of the promoter region and 1.388 bp of the downstream fragment originating from the start point of transcription (+1, G) in PG37 *lip*I were identified.

By computer-assisted analysis, it was revealed that 2 TTATTT sequences located at –576 bp and –40 bp upstream the transcription start site and 3 CAAT boxes existed in the promoter region of PG37 *lip*I. It was demonstrated that several gene promoters miss the classical TATA box. In this case, other consensus sequences such as TTATTT could act as substitutes for TATA box [18, 19]. A putative TATA box (–40 bp position) was in agreement with the consensus distances generally found in promoters. In eukaryote, functional CAAT box is typically found about –75 bp upstream the transcriptional start site. However, some CAAT boxes might locate further from the transcriptional start site [20]. PG37 *lip*I contains no

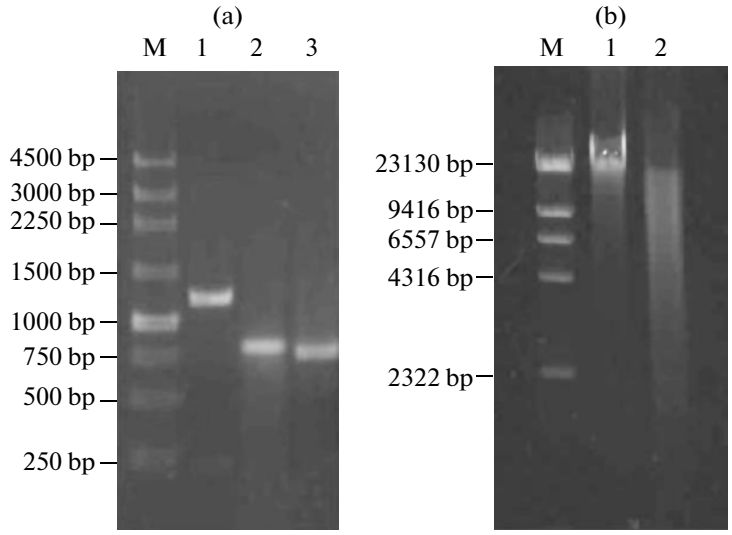

Fig. 2. Cloning of PG37 *lip*I from *P. cyclopium* PG37. (a) Amplification of the downstream fragment and promoter region of *lip*I from *P. cyclopium* PG37 by usual PCR and ligation-mediated PCR: M – 250 bp DNA marker; lane 1 – the usual PCR amplification of the downstream fragment of PG37 *lip*I; lane 2 – the first round of ligation-mediated PCR amplification of the promoter region of PG37 *lip*I; lane 3 – the second round of PCR amplification of the promoter region of PG37 *lip*I. (b) The genomic DNA of *P. cyclopium* PG37 and the DNA digested with restriction enzyme *Eco*RI: M, λ-*Hin*dIII digest DNA marker; lane 1 – PG37 genomic DNA; lane 2 – PG37 genomic DNA digested with restriction enzyme *Eco*RI.

CAAT box at about –75 bp position, although it does contain 3 CAAT boxes at –13, –384 and –590 bp, respectively. The CAAT at –384 or –590 bp of PG37 *lip*I was identified as putative CAAT box.

Bioinformatics analysis indicated that the promoter region possesses several putative transcription factor binding sites. 5 HSF (heat shock factor) binding sites (–5, –46, –242, –433 and –584 bp); four ADR1 (alcohol dehydrogenase gene regulator 1) binding sites (–16, –56, –272 and –437 bp); and several other *cis*-elements (data not shown) were found in PG37 *lip*I promoter region in response to various stresses. The fact that many *cis*-elements present in promoter region suggests that PG37 *lip*I is controlled by a complicated regulatory mechanism.

Amino acid sequence characterization of PG37 LipI. SignalP predicted an unambiguous signal peptide cleavage site between Ser^{20} and Ala^{21}, indicating PG37 LipI as a secretory protein. The preprolipase of 285 amino acid residues was predicted to contain a 20-aa signal peptide, a 7-aa propeptide and a 258-aa mature peptide [15]. Propeptide also exists in *Rhizopus oryzae* [21], *Fusarium heterosporum* [22] and many other microbial lipases. In fungal lipases, it has been shown that the propeptide that is normally removed from the lipase during its maturation eliminates the phospholipolytic activity of the proenzyme while increasing the lipolytic activity [1]. It was predicted by TMHMM that there is no obvious transmembrane domain in PG37 LipI. The hydrophobicity of PG37 LipI was analyzed using ProtScale. As shown in Fig. 4, the minimum and maximum hydrophobic values were – 2.389 and 2.478, respectively; and four major hydrophobic regions are MLFNYQSLLVGVSLISQALS (1–20 aa), VHRPWSAV (126–133 aa), AHVALA (168–173 aa) and AVTPGHIA (263–270 aa).

Molecular weight of 27291.8 Da and isoelectric point (pI) of 6.16 are calculated from the mature PG37 LipI, and its formula is $C_{1215}H_{1887}N_{323}O_{374}S_9$. The total number of negatively charged residues (Asp, Glu) is 22, while for the positively charged residues (Arg, Lys) it is 19. The hydrophobic residues (Ala, Ile, Phe, Leu, Met, Pro, Val and Trp) and uncharged residues (Asn, Cys, Gln, Gly, Ser, Thr and Tyr) occupy 44.9% and 35.1% of the total number, respectively. According to extremely low average of hydrophilicity (0.057), PG37 LipI is identified as a hydrophobic protein. Estimated instability index of 17.03 that is less than 40 and half-life of 4.4 h both suggest that PG37 LipI is a stable protein. Molecular weight of the native PG37 LipI was estimated to be about 29 kD by gel filtration using Sephadex G-150 and that of the denatured PG37 LipI was determined to be about 27.5 kD by its mobility on SDS-PAGE, which were almost in agreement with the calculated molecular weight. Nevertheless, the calculated pI is different from the pI (5.4) of native LipI determined by isoelectric focusing gels [15]. Post-translational modifications and higher structures of protein may account for the different pIs [23].

The result of motifs predicted by InterProScan showed that PG37 LipI contains a Lipase_3 motif and belongs to α/β hydrolase superfamily. Analyzed by Protfun, PG37 LipI was supposed to possess the function of fatty acid metabolism and amino acid biosynthesis.

−632 GAATTCATAA TGCTTAAATC ACGTATGGTA TAAGTCTATG CTCAATTCTC TAAATGATAT

CAAT box

−572 CTTTGCTTAT TTTATAGCTT ATTATATTTA AATTAGATAT TGAACAGCTA TTGAATCGTC

TATA box

−512 GTGCACGGAG CGGGTTAACG GTGAGCCAGG CCGTCGAAAT TTAATATGGG AAAATCCTCC

−452 GTACTACGGC CCCATTTCTG TGCTTCAAGA CATAAACATC GCCAAAGTGG TTCACGGGTA

−392 GTTATTAGCA ATTTTGATTA GTTCACGATG GCTTTCGGCA CATATGTCTC AGCGGCCTGG

CAAT box

−332 TCCCATACGG TGTGAAGATC AAATGAGGCT TTGAAGCCTA TAGGTAGTAC TTGCACTCGC

−272 TGGGGCTGTT ATCGCCATAC TGGTAAACCG AGAAATCCAC GGAGATTCCC CGAATGGTTC

−212 ATTTGTAAAT TACCTTGCAT GATGGCTTCC TGACTCTATA CTATGCATTA CCTGTTTGGG

−152 ATGTCATCCC CGACGCAGTC CACTTGGCGC CACATTCGGC CACCTTCCCT GCATTTAGTG

−92 TGCAAATATA GCGACAACGA GTACGATGGG ACTTTGAGGG GTACCTGAAG AATTATTTAA

+1

TATA box

−32 AGAGCAAGAT AACCCCACAC AATTTTCTGT TTGTGCATCC AAAGACAACG GTCCAGCAGC

CAAT box

+29 CTTCCCCAAG AGTATTCTAT AACACCCTCA TACATTGTTT CGAT***ATG***TTG TTCAACTACC

+89 AATCTTTACT CGTGGGAGTC TCTCTGATCT CTCAAGCCCT GTCTGCACCT ATTTTGGAGT

+149 CGAGGGCAAC TGCTGGTATG TCAATTTATC AGCCCAGCTC CATTCGACTA GGCAGCTCTA

+209 ACATTTTCTC AAGACGCCGC TGCCTTCCCT GATCTGCACC GTGCAGCAAA GCTTTCTTCC

+269 GCTGCCTACA CAGGTTGCAT CGGAAAGGCC TTCGATGTCA CTATCACCAA GAGGATTTAT

+329 GACCTCGTGA CCGACACCAA TGTAGGCGTG GTATTTGCCC TATACGTTGG CAAATAACTA

+389 ATTTCAATAG GGATTCGTCG GATACTCCAC CGAGAAGAAG ACCATCGCGG TCATCATGAG

+449 GGGCTCGACT ACCAGTATGG CAAAATCACT TCTTAATACT AGCTCAACAT GCTAATTAAT

+509 CCAGTCACCG ACTTCGTGAA CGACATTGAC ATTGCTCTCA TCACTCCTGA GCTCTCGGGC

+569 GTGACTTTCC CCTCTGATGT GAAGATCATG AGAGGTGTTC ACAGACCTTG GTCCGCTGTA

+629 CACGACACCA TCATTACTGA AGTCAAGGCT CTCATTGCGA AGTACCCTGA TTACACTCTG

+689 GAAGCAGTCG GACATTCCCT CGGTGGTGCC CTCACATCCA TTGCCCACGT TGCCCTGGCC

+749 CAGAACTTCC CGGACAAGTC ACTTGTCAGC AATGCCCTTA ACGCCTTCCC CATCGGCAAC

+509 CAAGCGTGGG CCGACTTTGG TACTGCGCAG GCCGGTACCT TCAACCGCGG AAATAACGTT

+869 CTTGACGGTG TCCCTGTAAG CCATGGTTGA AACTGACATG CTATATTAAA GTAGCTAACC

+929 ATACAACACA GAACATGTAC TCGAGCCCGC TTGTTAACTT CAAGCACTAT GGAACCGTGA

+989 GTTATCCTAC CGATTCCCCT TTAGATATCA ATGTTCAATC GCTTATCAAC CATAACCATG

+1049 CACAGGAATA CTACAGCTCT GGTACCGAGG CTAGCACCGT GAAGTGCGAA GGCCAGCGTG

+1109 ACAAGTCTTG CTCTGCCGGC AATGGCATGT ACGCTGTCAC TCCCGGTCAC ATCGCAAGCT

+1169 TTGGCGTCGT GATGCTTACT GCTGGTTGTG GCTATCTGAG C***TGA***GTACCA GAAGCGAATA

+1229 TGACAGCAAT GTTCGACACA GATCACTTAT TCTGAGGACA GAGCGACCTC GGATAACTAA

+1289 TATATTATCT ACTGTTCTAA TAGTTAGTTT ATACTTATTT GTGTATATTG GATGGTTGGT

+1349 CATCCAAAGC GATCAGCGT**A ATAAA**AATAC ATCTGATTTG

Fig. 3. Nucleotide sequence of PG37 *lip*I. DNA sequences of introns are shown in gray shadows. The letters in boxes, TTATTT and CAAT, indicate the putative TATA box and CAAT box, respectively; the letter G marked with "+1" indicates the starting point of transcription; the start codon and stop codons are shown in italic; the polyadenylation signal, AATAAA, is shown as underlined letters.

BLAST analysis of PG37 LipI showed 32, 30, 28 and 26% identity with lipases of *A. parasiticus* (GenBank: AAO17920), *P. camembertii* (PDB: 1TIA), *T. lanuginosus* (Swiss-Prot: O59952) and *R. miehei* (Swiss-Prot: P19515), respectively. The result of alignment showed that the pentapeptide sequence, G-H-S-L-G, in 5 lipases mentioned above is identical, with the consensus sequence in PG37 LipI locating at from 157 to 161 residue (Fig. 5). A catalytic triad, Ser^{132}-Asp^{188}-His^{241} which exists in almost all lipases, was also found in PG37 LipI.

Structure analysis of PG37 LipI. The secondary structure was analyzed by SOPMA and the result showed that the PG37 LipI contains 31.78% α-helix, 19.77% extended strand, 5.81% β-turn, and 42.64% random coil. The α-helix and random coil constitutes the main part of the secondary structure. *P. camemberti* lipase crystal structure (PDB: 1TIA) was selected as a suitable template by a BLAST search. The tertiary structure of PG37 LipI was predicted in EsyPred3D Protein Modelling Server (Fig. 6). The core structure of PG37 LipI, where β-sheets are surrounded by α-helices, is in accordance with α/β hydrolase fold.

Although the overall homology between lipases is low, all known lipases share a comparable tertiary structure which is common to the α/β hydrolase family [21]. In most lipase structures, the active center, Ser-Asp-His, is inaccessible due to coverage by a flexible alpha helical lid (residues Thr^{66} to Val^{72}). The lid would most likely be involved in a conformational change that allows the substrate access to the active site [24]. These studies provided a structural basis for the well-known phenomenon of interfacial activation. On the contrary, Karkhane et al. [25] used site-directed mutagenesis to introduce mutation that Phe^{181} was substituted with alanine in the lid domain of *Bacillus thermocatenulatus* lipase. F181A mutation increased the distance between Phe^{181} and catalytic Ser^{114}, which led to a decrease in steric hindrance. F181A mutation increased overall lipase activity by up to 2.6-fold (4670 U/mg) toward C8 substrate. It also resulted in optimal lipase activity at 65°C rather than 55°C.

In this study, we reported the cloning of the complete DNA gene encoding an alkaline lipase of *P. cyclopium* and bioinformatics analysis of the DNA sequence of PG37 *lip*I and its deduced amino acid sequence. The elucidation of the primary structure of the lipase of *P. cyclopium* with bioinformatics approaches provided us with a solid basis not only for the regulation and expression of PG37 *lip*I, but also for PG37 LipI rational modification.

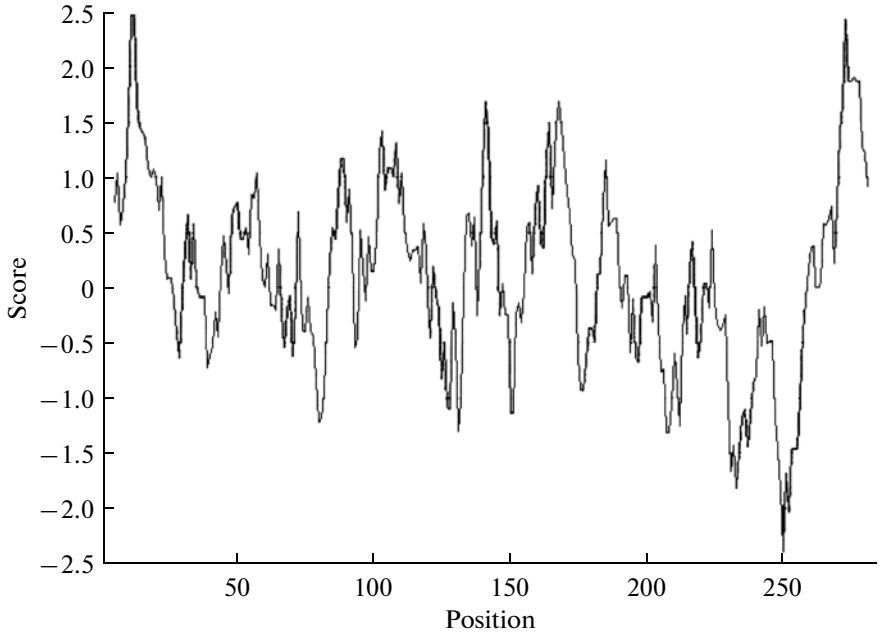

Fig. 4. Prediction of hydrophobicity by ProtScale.

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APAL    ..............................................................MRFLSGFVSVLSSVA  15
PCAL    ...............................................................................  0
PCYL    ...............................................................................  0
RMIL    MVLKQRANYLGFLIVFFTAFLVEAVPIKRQSNSTVDSLPPLIPSRTSAPSSSPSTTDPEAPAMSRNGPLPSDVETKYGMA  80
TLAL    ..............................................................MRSSLVLFF  9
Consensus

APAL    LLGYAYPTAIDVRDIPTTQLEDFKFWVQYAAATYCPNNYVAKDGEKLNCSVGNCPDVEAADSTVKLSFSDDTITDTAGFV  95
PCAL    ............DVSTSELDQFEFWVQYAAASYYEADYTAQVGDKLSCSKGNCPEVEATGATVSYDFSDSTITDTAGYI  67
PCYL    .MLFNYQSLLVGVSLISQALSAPILESRATADAAAFPDLHR..AAKLSSAAYTGCIGKAFDVTITKRIYD.LVTDTNGFV  76
RMIL    LNATSYPDSVVQAMSIDGGIRAATSQEINELTYYTTLSANSYCRTVIPGATWDCIHCDATEDLKIIKTWSTLIYDTNAMV  160
TLAL    VSAWTALASPIRREVSQDLFNQFNLFAQYSAAAYCGKNNDAPAGTNITCTGNACPEVEKADATFLYSFEDSGVGDVTGFL  89
Consensus                                                                       d

APAL    AVDNTNKAIVVAFRGSYSIRNWVTDATFPQTDP.....GLCDGCKAELGFWTAWKVSRDRIIKTLDELKPEHSDTKIVVV  170
PCAL    AVDHTNSAVVLAFRGSYSVRNWVADATFVHTNP.....GLCDGCLAELGFWSSWKLSRDDIIKELKEVVAQNPNYELVVV  142
PCYL    GYSTEKKTIAVIMRGSTTITDFVNDIDIALITPELSGVTFPSDVKIMRGVHRPWSASHDTIITEVKALIAKYPDYTLEAV  156
RMIL    ARGDSEKTIYIVFRGSSSIRNWIADLTFVPVSY.....PPVSGTKVHKGFLDSYGPDSQNELVATVLDQFKQYPSYKVAVT  235
TLAL    ALDNTNKLIVLSFRGSRSIENWIGNLNFDLKEIN....DICSGCRGHDGFTSSWRSVADTLRQKVEDAVREHPDYRVVFT  165
Consensus               rgs                              g       v          y

APAL    GHSLGAAIASLAAADLR....TKNY.DAILYAYAAPRVANKPLPEFITNQ..GNNYRFTHND.DPVPKLPLLTMG.YVHI  241
PCAL    GHSLGAAVATLAATDLR....GKGYPSAKLYAYASPRVGNAALPKYITAQ..GNNFRFTHTN.DPVPKLPLLSMG.YVHV  214
PCYL    GHSLGGALTSIAHVALAQ...NFPDKSLVSNALNAFPIGNQAWTDFGTAQ..AGTFNRGNNVLDGVPNMYSSPLVNFKGY  231
RMIL    GHSLGGATALLCALDLYQREEGLSSSNLFLYTQGQPRVGDPAFANYVVST..GIPYRRTVNERDIVPHLPPAAFG.FLPA  312
TLAL    GHSLGGALATVAGADLR....GNGY.DIDVFSYGAPRVGNRAFAEFLTVQTGGTLYRITHTN.DIVPRLPPREFG.YSHS  238
Consensghslg    a       l                   a                   d  vp          h

APAL    SPEYYITAPDNTTVTDNQVTVLGGYVNFEGNTGTSGGLPDLLAFHSSVWYFIHADACKGHGLPFR.  306
PCAL    SPEYWITSPNNATVSTSDIKVIDGDVSFDGNTGT..GLPLLTDFEAHIWYFVQVDAGKGPGLPFKR  278
PCYL    GTEYYSSGTEASTVKCEGQRDKSC......SAGN....GMYAVTPGHIASFGVVMLTAGCGYLS..  285
RMIL    GEEYWITDNSPETVQVCTSDLETS......DCSN..SIVPFTSVLDHLSYFGINTGLCT.......  363
TLAL    SPEYWIKSGTLVPVTRNDIVKIEG...IDATGGN..NQPNIPDIPAHLWYFGLIGTCL........  291
Consensusey             v                                   h    f
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Fig. 5. Alignment of lipases from *A. parasiticus* (APAL), *P. camembertii* (PCAL), *P. cyclopium* (PCYL), *R. miehei* (RMIL) and *T. lanuginosus* (TLAL). The identical amino acid residues are highlighted in shadow.

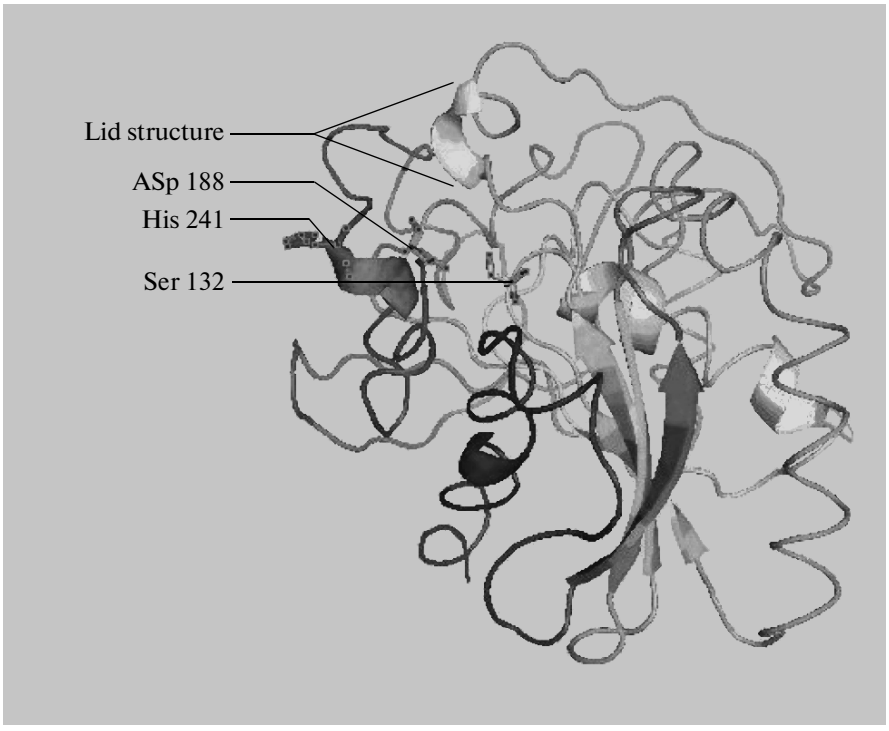

Fig. 6. Prediction of the tertiary structure of PG37 LipI. Ser^{132}-Asp^{188}-His^{241}, three amino acid residues compose the active center in the tertiary structure. The alpha helical lid structure was marked.

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