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Monoacylglycerol Lipase from Moderately Thermophilic *Bacillus* sp. Strain H-257: Molecular Cloning, Sequencing, and Expression in *Escherichia coli* of the Gene[1]

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Monoacylglycerol lipase [MGLP, EC 3.1.1.23] is produced intracellularly by the moderately thermophilic *Bacillus* sp. strain H-257. The gene encoding MGLP was cloned, sequenced, and expressed in *Escherichia coli*. A genomic library of *Bacillus* sp. strain H-257, prepared in the plasmid vector pACYC184, was screened with a 0.2-kbp DNA fragment amplified by the polymerase chain reaction (PCR) with oligonucleotide primers designed based on the amino acid sequence of a purified MGLP. The plasmid pMGLP31, identified by hybridization with the amplified DNA fragment, contained a 5.3-kbp insert from *Bacillus* sp. strain H-257 DNA. Sequence analysis of the MGLP gene revealed an open reading frame encoding MGLP consisting of 250 amino acids, with a calculated molecular mass of 27.4 kDa. The deduced amino acid sequence of MGLP contained the consensus pentapeptide (-Gly-Xaa-Ser-Xaa-Gly-), which is conserved among lipases, esterases, and serine proteases. The MGLP is homologous to a putative esterase/lipase from *Streptomyces coelicolor* (41.8% homology). When pMGLP31 was introduced into *E. coli* DH1, the transformants produced MGLP intracellularly as an active form to an approximately 13.8-fold greater extent than *Bacillus* sp. strain H-257. The purified recombinant MGLP was shown to be identical to the native enzyme in terms of chromatographic behavior, isoelectric point, and physicochemical and catalytic properties.

Key words: amino acid sequence, DNA sequencing, molecular cloning, monoacylglycerol lipase, thermostable enzyme.

Lipase [triacylglycerol lipase, EC 3.1.1.3] is useful in various fields of industry as a biocatalyst and has been used widely for a long time. In this category of lipases, di- or monoacylglycerol lipases that hydrolyze partial-acylglycerols as substrates have been reported (*1–8*). Monoacylglycerol lipase, which catalyzes the hydrolysis of monoacylglycerols and produces glycerol and free fatty acids, is distributed in animals (*9–11*) and microorganisms (*12*, *13*). Many of the previously reported enzymes, however, also hydrolyze di- and triacylglycerols (*14*, *15*). Until now, only limited information on a specific monoacylglycerol-hydrolyzing enzyme, monoacylglycerol lipase [MGLP, EC 3.1.1.23], has been available, and MGLP from bacteria has not yet been described. We have purified the MGLP produced by the moderately thermophilic *Bacillus* sp. strain H-257 and reported its enzymatic properties (*16*). This MGLP hydrolyzes monoacylglycerols but not di- and triacylglycerols to an appreciable extent and is thermostable up to 60°C. It should be useful for analytical purposes such as the measurement of serum lipase levels and analysis of monoacylglycerols in food. However, MGLP production by *B.* sp. strain H-257 is low (about 1.6 ng/ml). The aim of this study was to increase the production level by the methods of molecular cloning, sequence the gene encoding MGLP from *B.* sp. strain H-257, and express the gene in an *Escherichia coli* host-vector system. In this paper, we report (i) the nucleotide sequence of the MGLP gene from *B.* sp. strain H-257 and the deduced amino acid sequence, (ii) the production of the MGLP expressed in *E. coli*, and (iii) several characteristics of the enzyme.

MATERIALS AND METHODS

Bacterial Strains and Plasmids—*B.* sp. strain H-257 isolated from soil in Kagoshima prefecture was used as a source of MGLP and chromosomal DNA. *E. coli* DH1 (F⁻ *recA*1 *endA*1 *gyrA*96 *thi*-1 *hsdR*17 *supE*44 *relA*1) and plasmid pACYC184 (*17*) were used to construct the genomic libraries. *E. coli* MV1184 (*ara* Δ(*lac-proAB*) *rpsL thi*

[1] The nucleotide sequence of MGLP reported in this paper at positions −137 to 829 has been submitted to the DDBJ, EMBL, and GenBank databases under the accession number E05047. Nucleotide "T" at position of 581 in E05047 should be changed to nucleotide "C."

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Abbreviations: *B.* sp., *Bacillus* sp.; GK, glycerol kinase; GPO, glycerol-3-phosphate oxidase; MGLP, monoacylglycerol lipase; TOOS, *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-*m*-toluidine.

(ϕ80d*lacZ*ΔM15) Δ(*srl-recA*)306::Tn10(*tet*^{r}) F$'$ [*traD*36 *proAB*$^+$ *lacI*^{q} *lacZ*ΔM15]) and plasmids pUC118 and pUC119 (*18*) were used for nucleotide sequencing.

Media—The cultivation medium for *B.* sp. strain H-257 has been reported previously (*16*). Brain heart infusion (BHI) medium and agar (Difco Laboratories, Detroit, MI, USA) supplemented with 30 μg/ml chloramphenicol were routinely used in the cloning experiments.

Purification of MGLP—MGLP was purified according to the method described previously (*16*). In brief, cells were collected from the culture by centrifugation. The cells were suspended in 10 mM potassium phosphate buffer (pH 7.0) containing lysozyme to prepare a cell-free extract. Then, the MGLP was purified using a combination of affinity chromatography and gel filtration chromatography columns including Octyl-Sepharose CL-4B, Q-Sepharose FF, and Superose 12 (Pharmacia, Uppsala, Sweden).

Synthesis of Oligonucleotides—Based on the amino acid sequences of the purified MGLP with and without treatment with lysyl-end peptidase, two sets of oligomers were designed and synthesized by the phosphoamidite method using a Cyclone Plus DNA Synthesizer (MilliGen Bioresearch, Bedford, MA, USA). These oligomers were used as primers for PCR.

Preparation of a Probe for Screening of MGLP-Positive Clones—The probe for the hybridization experiments was prepared according to the PCR method (*19*) with a commercially available kit (Perkin-Elmer Cetus, Foster City, CA, USA). The primers were applied to a DNA Thermal Cycler (Perkin-Elmer Cetus) with the following thermocycle program: 1 min at 94°C, followed by 35 cycles of 2 min at 55°C and 3 min at 72°C, with a final extension at 72°C for 15 min. A major product of 0.2-kbp was isolated from the agarose gel after electrophoresis. The fragment was used as a DNA probe for genomic Southern and colony hybridization experiments.

Construction of Genomic Libraries—Most of the methods used for molecular cloning were based on those of Maniatis *et al.* (*20*). *B.* sp. strain H-257 cells were prepared as described previously (*16*). Chromosomal DNA of *B.* sp. strain H-257 was prepared according to the method of Saito and Miura (*21*). The DNA was completely digested with *Cla*I, and then the DNA fragment (about 5.3 kb) obtained from the agarose gel after electrophoresis was ligated to the dephosphorylated *Cla*I site in pACYC184 and introduced into *E. coli* DH1.

Screening of MGLP-Positive Clones—Transformants were selected as colonies on agar plates in BHI medium containing 30 μg/ml of chloramphenicol. Screening of MGLP-positive clones was carried out principally as described by Davies *et al.* (*22*). A Magnagraph Nylon Membrane (Micron Separations Inc., Westboro, MA, USA) layered on the bacterial colonies was treated with 0.5 N NaOH containing 1.5 M NaCl. After the membrane was neutralized with 0.5 M Tris-HCl (pH 7.0) containing 0.3 M NaCl, DNA was fixed on the membrane at 80°C for 2 h. The DNA probe prepared by PCR (0.2 kb) was labeled with 370 kBq of [γ-^{32}P]ATP (Amersham, Buckinghamshire, England). DNA hybridization was carried out overnight at 42°C and clones with strong signals were selected.

Nucleotide Sequence of the MGLP Gene—The 1.9-kbp insert DNA of recombinant plasmid pMGLP31 was subcloned into pUC118 and pUC119 and sequenced by the dideoxy-chain termination method (*23*) using [α-^{32}P]dCTP and a 7-DEAZA sequencing kit (Takara Shuzo, Kyoto). Computer analysis of the DNA sequence and the deduced amino acid sequence was performed with GENETYX-WIN (SDC Software Development, Tokyo).

Production of MGLP in E. coli—*E. coli* DH-1 carrying pMGLP31 was cultured in BHI medium containing 30 μg/ml of chloramphenicol. The cells were cultured overnight at 37°C on a rotary shaker (60 rpm), collected by centrifugation (7,000 ×*g* for 5 min), suspended in the same volume of 10 mM Tris-HCl (pH 7.0), and ultrasonicated. The supernatant was obtained by centrifugation (7,000 ×*g* for 5 min) after heat treatment at 60°C for 30 min. This solution was used as a cell-free extract and MGLP productivity was determined.

Assay of MGLP Activity—The MGLP activity was routinely measured according to the method described previously (*16*). In brief, the reaction mixture, containing 100 μl of 0.2 M PIPES-NaOH (pH 7.3), 50 μl of 0.3% 4-aminoantipyrine, 50 μl of 0.2% TOOS, 50 μl of 45 U/ml peroxidase, 25 μl of 20 mM $MgCl_2$, 25 μl of 20 mM ATP, 10 μl of 25 U/ml GK, 15 μl of 1,000 U/ml GPO and 75 μl of H_2O, and 50 μl of substrate solution (10 mM monolauroylglycerol containing 0.5% Triton X-100), was kept at 37°C, and the reaction was started by the addition of the enzyme solution (50 μl). After incubation at 37°C for 10 min, the reaction was stopped by adding 2.5 ml of 0.5% SDS solution. The glycerol generated was determined from the absorbance at 550 nm. One unit (U) of MGLP activity was defined as the amount of enzyme that liberates 1 μmol of glycerol per min at 37°C.

RESULTS AND DISCUSSION

In our previous study, the amino acid sequence of the N-terminal region of MGLP was identified (*16*). We next planned to clone the MGLP gene using a DNA fragment amplified by PCR with chromosomal DNA and oligonucleotide primers designed based on the amino acid sequence of purified MGLP.

Cloning and Sequencing of the MGLP Gene—The purified MGLP obtained from *B.* sp. strain H-257 was digested with lysyl-end peptidase. The amino acid sequences of the resulting fragments and the amino (N)-terminals of the protein were analyzed by the Edman degradation method. The sequences of the N-terminal of the undigested MGLP and its internal peptides are shown in Fig. 1. The following two sets of 20-nucleotide-long mixed oligomers were prepared as primers for PCR:

[N-terminal (Gly^9–Ala^{15}): 5′-GGIGCIGARCCITTYTAYG-C-3′ (sense strand)]

[K-6 (Thr^{13}–Ala^{19}): 5′-GCIACCCARTCRTGRAAIGT-3′ (antisense)],

where R is G or A; Y is T or C; and I is inosine; fitting with all bases.

The 0.2-kbp PCR product was isolated from the agarose gel and used as a probe for genomic Southern analysis. Only one main signal (about 5.3 kb) was detected in the genomic Southern hybridization blot when the chromosomal DNA was digested completely with the restriction enzyme *Cla*I (data not shown).

The genomic library was distributed on BHI medium

N-terminal	S - E - Q - Y - P - V - L - S - G - A -
	E - P - F - Y - A - E -
Internal	
K-6	G - H - G - T - H - Y - E - D - M - E -
	R - T - T - F - H - D - W - V - A - S -
	V - E - E - G - Y - G - W - L - K -
K-9a	E - I - V - R - L - R - N - S - Y - H -
	V - A - T - L - D - Y - D - Q - P - M -
	I - I - E - R - S - L - E - F - F - A -
	K -
K-13	Q - R - ? - Q - T - I - F - V - T - G -
	L - S - M - G - G - T - L - T - L - Y -
	L - A - E - H - H - P - D - I - ? - G -
	I - V - P - I - N -

Fig. 1. **Amino acid sequences of N-terminal and internal polypeptides obtained from the purified MGLP.** K-6, K-9a, and K-13 are the peptide fragments digested by lysyl-end peptidase. Question marks indicate amino acids that could not be identified.

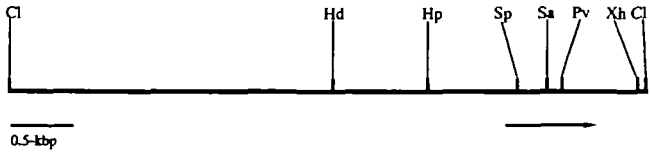

Fig. 2. **Restriction map of the 5.3-kbp DNA fragment containing the MGLP gene from *Bacillus* sp. strain H-257.** The arrow indicates the genomic region covering the MGLP gene and the direction of transcription. Abbreviations: Cl, *Cla*I; Hd, *Hin*dIII; Hp, *Hpa*I; Sp, *Sph*I; Sa, *Sal*I; Pv, *Pvu*II; Xh, *Xho*I.

agar plates containing 30 μg/ml of chloramphenicol (approximately 1,000 colonies per plate), and colony hybridization was carried out. Positive clones were obtained in a population of about 27,000 colonies. The plasmid isolated from the clones had a 5.3-kbp *Cla*I insert in the same site of pACYC184. The restriction map of the *Cla*I fragment from *B.* sp. strain H-257, contained in the recombinant plasmid designated as pMGLP31, is shown in Fig. 2.

The *Cla*I fragment (5.3 kb) was subcloned into pUC118 and pUC119, and then the MGLP gene located in the *Hpa*I–*Xho*I fragment (1.9 kb) was sequenced by the dideoxy-chain termination method. The nucleotide sequence of the MGLP gene, as well as the 5′ and 3′ adjacent regions, is shown in Fig. 3. An open reading frame consisting of 753-bp starts at ATG (A=+1) and ends at TAA (T=+751) and is predicted to encode a protein with a calculated molecular mass of 27.4 kDa. The sequence of the MGLP gene has a GC content of 57.4%. The amino acid sequence of the N-terminal of purified MGLP is similar to the translated protein sequence of the MGLP gene. Also, the K-6, K-9a, and K-13 peptides are identical in the same reading frame. These findings show that the nucleotide sequence of this reading frame is that of the gene encoding MGLP.

Analysis of the nucleotide sequence revealed a potential consensus Shine-Dalgarno sequence in the nucleotide sequence upstream of the translational initiation codon, ATG, and two inverted repeat sequences were observed downstream of the stop codon, TAA, of the reading frame. Four candidates for promoters were found as shown in Fig. 3, from −689 to −684, from −680 to −675, from −648 to −643, and from −492 to −487 and from −666 to −661, from −657 to −652, from −625 to −620, and from −469 to −464, respectively.

The genes encoding mono- and diacylglycerol lipases from *Aspergillus oryzae* IFO-4202 (*24*) and from *Penicillium camembertii* U-150 (*25*) were cloned and sequenced previously. We compared the translated protein sequence of the MGLP from *B.* sp. strain H-257 with these enzymes. However, no significant homology was observed. The sequence of MGLP was also compared with the sequences deposited in the DDBJ database using the FASTA search program. Figure 4 shows alignment of the amino acid sequence of MGLP with those of *Streptomyces coelicolor* (*26*) and *B. stearothermophilus* (*27*) esterases. The amino acid sequence of MGLP shows 41.8 and 33.3% homology to the sequences of the esterases of *S. coelicolor* and *B. stearothermophilus*, respectively.

Sequence analysis of lipases from a diverse array of bacteria has shown that these enzymes contain a serine protease-like catalytic triad consisting of Ser, His, and Asp residues (*28*). The Ser residue, one of the active site residues, is located within a semi-conserved pentapeptide, either Gly(Ala)-Xaa-Ser-Xaa-Gly or Gly-Xaa-Ser-Xaa-Ser, where Xaa indicates any amino acid. We found the sequence -Gly-Leu-Ser-Met-Gly- conserved in MGLP at position 95 (Fig. 3). Ser^{97} thus appears to be the active serine residue. However, we could not identify two other active site residues (His and Asp) in MGLP because of the low sequence similarity between MGLP and other lipases whose active site residues have been identified. The HG dipeptide found in many lipases, the 70–100 amino acid sequence N-terminal to the catalytic site serine, was also found at position 27. Detailed structural comparison awaits elucidation of the three-dimensional structure of MGLP, which is under investigation.

Production of MGLP in B. sp. Strain H-257 and in E. coli Carrying pMGLP31—*B.* sp. strain H-257 were cultured and a cell-free extract was obtained according to a method described previously (*16*). *E. coli* DH1 carrying pMGLP31 was grown aerobically overnight at 37°C in 1.5 ml of BHI media containing 30 μg/ml of chloramphenicol. A cell-free extract was also obtained by the same method coupled with heat treatment, which is effective in removing impurities derived from the host cells. The specific activity of the cell-free extract of *E. coli* DH1 carrying pMGLP31 was 13.8-fold greater than that of *B.* sp. strain H-257, whereas no MGLP activity was detected in *E. coli* DH1 cells carrying neither pMGLP31 nor vector plasmid pACYC184 alone (Table I).

The genes encoding the lipase from genus *Bacillus* have been cloned, sequenced and expressed in *E. coli*. Kim *et al.* reported a thermostable lipase from *B. stearothermophilus* L1 (*29*). The lipase expressed in *E. coli* has a molecular mass of 43 kDa and is stable up to 60°C. However, the expressed lipase appears to inhibit the growth of fast-growing *E. coli* host cells. The lipase is homologous to the thermoalkalophilic lipase from *B. thermocatenulatus* (94% identity). This thermoalkalophilic lipase reported by Rúa *et al.* (*30*), on the other hand, is weakly expressed in *E. coli* with expression plasmids containing the gene encoding either the precursor or the mature part under the control of the native promoter. A high level of expression was achieved when the *E. coli* OmpA signal sequence was placed immediately upstream of the mature part of the lipase gene to secrete the lipase into the periplasm of *E. coli*. In the case

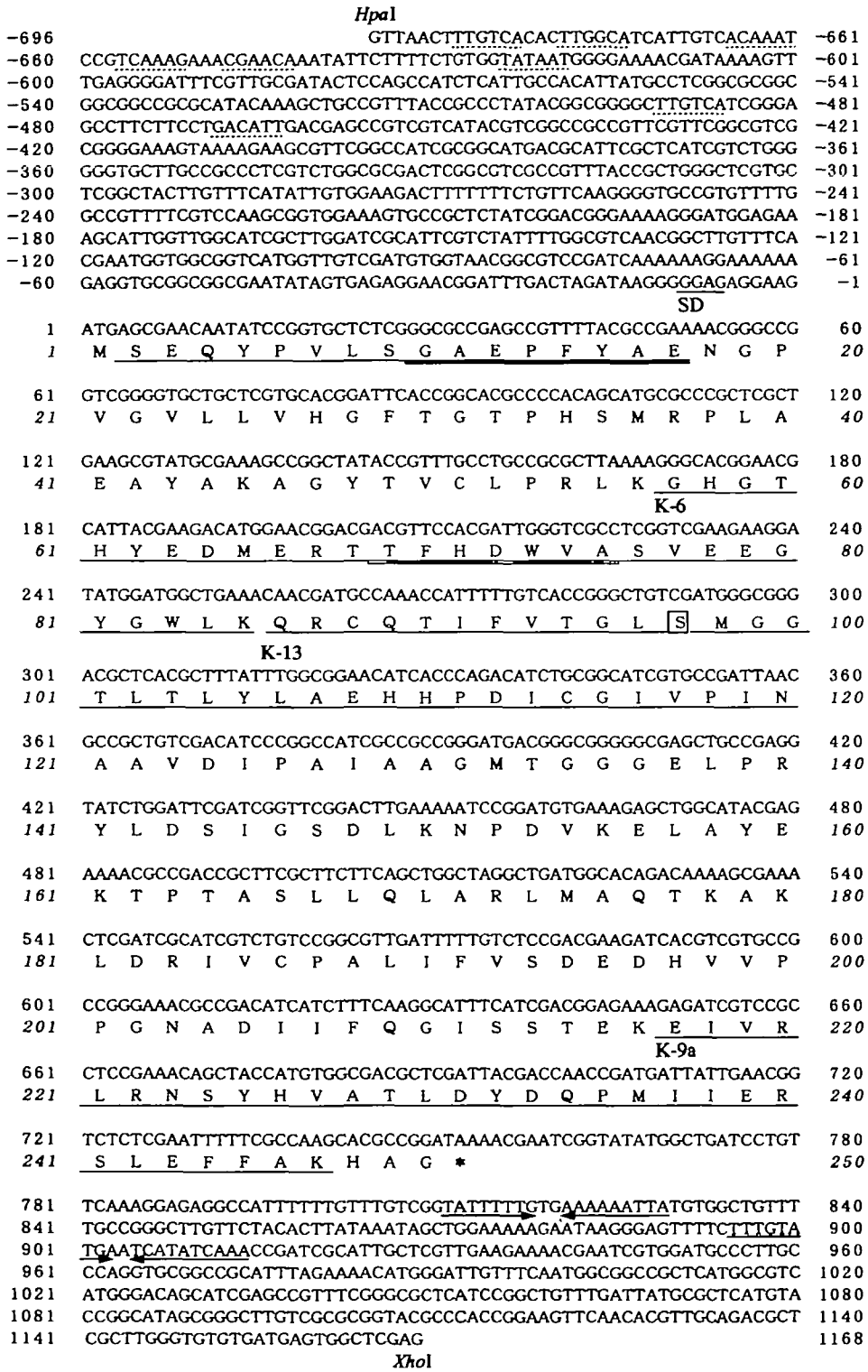

Fig. 3. **Nucleotide sequence and the deduced amino acid sequence of the MGLP gene from *Bacillus* sp. strain H-257.** The numbering of nucleotides starts at the 5′ terminus of the MGLP gene. Amino acid numbering is shown beneath the nucleotide numbers. Four candidates for promoters are dotted underlined. The sequences complementary to the oligomers for PCR are double underlined. The amino acid sequence of peptides isolated after lysyl-end peptidase partial digestion and the N-terminal of the undigested MGLP are underlined. The asterisk indicates the stop codon. The arrows indicate inverted repeat sequences. The box shows the Ser^{97} possible active site.

of MGLP, the gene was expressed well in a common *E. coli* host-vector system without any extra signal sequence.

Some *Pseudomonas* lipases require an accessory gene encoding a molecular chaperone located downstream of the lipase gene for efficient secretion and folding of active lipase (*28*). Various names have been used to designate the chaperones from various species, *e.g.* LipB, LipH and LimA for the chaperones from *Burkholderia glumae*, *P. aeruginosa*, and *Burkholderia cepacia*, respectively. When the lipase gene is over-expressed in *E. coli* without an accessory gene, the lipase is obtained as enzymatically inactive inclusion bodies. This is why the expression of these lipase genes is hampered in *E. coli*. In contrast, MGLP does not require a chaperone for folding of the active lipase.

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Mglp    1:MSEQYPVLSGAEPFYAENGPVGVLLVHGFTGTPHSMRPLAEAYAKAGYTVCLPRLKGHGT  60
S-est   1:----MSVLPGAEPFRHEGGDVGVLLCHGFTGSIQSLRPWADYLAARGLTVSLPLLPGHGT  56
B-est   1:---MTKIVP-PKPFFFDAGERAVLLLHGFTGNSADVRMLGRFLESKGYTCHAPIYKGHGV  56

Mglp   61:HYEDMERTTFHDWVASVEEGYGWLKQRCQTIF-VTGLSMGGTLTLYLAEHHPD-ICGIVP  118
S-est  57:RWQDEQVTGWQDWYAPVDRELRALRERCERVF-VAGLSMGGALALRLAAKFGLAVSGVVV  115
B-est  57:PPEELVHTGPDDWWQDVMNGYEFLFNKGYEKIAVAGLSLGGVFSLKLGYT-V-PIEGIVT  114

Mglp  119:INAAVDIPAIAA-GMTGGGELPNYLDSIGSDLKNPDVKEL-AYEKTPTASLLQLARLMAQ  176
S-est 116:VNPANRMHGVAQHALPVLRHLVPATKGLASDIAKPLSTEL-GHDRVPLHSAHSLRAFFRL  174
B-est 115:M-CAPMYIKSEETMYEGVLEYAREYKKREGKSEEQIEQEMEKFKQTPMKTLKALQELIAD  173

Mglp  177:TKAKLTRIVCPALIFVSDEDHVVPFGNADIIFQGISSTEKEIVRLRNSYHVATLDYDQPM  236
S-est 175:ADGDLPQVTQPLLLLRSPQDHVVPPADSARILGRVSSSDVTEILLEQSYHVATLDHDADR  234
B-est 174:VRDHLDLIYAPTFVVQARHDEMINPDSANIIYNEIESPVKQIKWYEQSGHVITLDQEKDQ  233

Mglp  237:IIERSLEFFAK-HAG------------------                            250
S-est 235:LFAESVAFIGRLAPLSVGEPESGLGKEGTAAGG                            267
B-est 234:LHEDIYAFLESLDW------------------                             247
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Fig. 4. **Alignment of the amino acid sequence of MGLP with other sequences.** Black boxes with white letters indicate common residues between MGLP and at least one other esterase at the given positions. Abbreviations: S-est, *Streptomyces coelicolor* putative esterase/lipase (accession No. AL109661) (*26*); B-est, *Bacillus stearothermophilus* IFO-12550 esterase (accession No. D12681) (*27*).

TABLE I. **The MGLP activity produced by *Bacillus* sp. strain H-257 and *E. coli* DH1 carrying pMGLP31.** Details are described in the text.

Strain	Specific activity (U/mg)
Bacillus sp. strain H-257	0.04
E. coli DH-1 carrying pMGLP31	0.55
E. coli DH-1	0
E. coli DH-1 carrying pACYC184	0

TABLE II. **Enzymatic properties of native and recombinant MGLPs.**

Property	Native MGLP	Recombinant MGLP
Optimum temperature	75°C	75°C
Optimum pH	pH 6–8	pH 6–8
pH stability	pH 7–10	pH 7–10
Thermostability	<60°C	<60°C
Isoelectric point	4.66	4.70
Specific activity	U/mg	U/mg
1-Monocapryloylglycerol ($C_{8:0}$)	113.8	113.2
1-Monolauroylglycerol ($C_{12:0}$)	121.1	120.8
1-Monomyristoylglycerol ($C_{14:0}$)	102.3	102.8
1-Monoparmitoylglycerol ($C_{16:0}$)	76.9	74.3
1-Monostearoylglycerol ($C_{18:0}$)	55.1	53.9
1-Monooleoylglycerol ($C_{18:1}$)	69.0	65.7
1-Monolinoleoylglycerol ($C_{18:2}$)	90.8	91.8
1,2-Dilinoleoylglycerol ($C_{18:2}$)	0	0
Trioleoylglycerol ($C_{18:1}$)	0	0
p-Nitrophenyl butylate ($C_{4:0}$)	28.9	27.2
p-Nitrophenyl caplylate ($C_{8:0}$)	15.4	15.9

Enzymatic Properties of the MGLP Expressed in E. coli—Both native and recombinant MGLPs were purified from cell cultures. The chromatographic behavior of recombinant MGLP was similar to that of native MGLP (data not shown). The enzymatic properties of these purified enzymes were determined and are summarized in Table II. The optimum temperature, optimum pH, pH stability, thermostability, and isoelectric point of recombinant MGLP are identical to those of native MGLP. Furthermore, the substrate specificity is identical or very similar. SDS-PAGE was carried out in a 12.5% polyacrylamide gel. As shown in Fig. 5, the mobility of the recombinant MGLP is the same as that of native MGLP. These results indicate that the recombinant MGLP is identical to the native enzyme.

In this study, the amino acid sequence of MGLP produced by moderately thermophilic *B.* sp. strain H-257 and

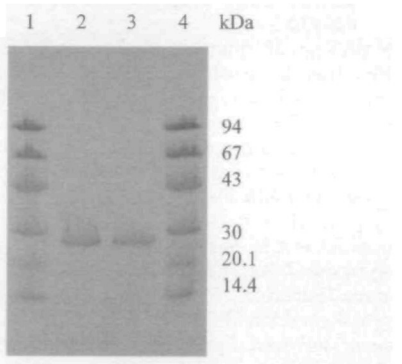

Fig. 5. **SDS-PAGE of native and recombinant MGLPs.** Lanes 1 and 4, molecular marker proteins including phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa); lane 2, native MGLP; lane 3, recombinant MGLP.

the nucleotide sequence of the gene encoding MGLP were detected for the first time, and the MGLP gene was successfully expressed in a common *E. coli* host-vector system. *E. coli* DH1 carrying pMGLP31 produce MGLP intracellularly as the active form to an approximately 13.8-fold greater extent than *B.* sp. strain H-257. This enzyme was shown to be identical to the native enzyme.

We established an effective procedure for the production of this enzyme using a molecular biological technique. There is the possibility of an exchange or modification of the substrate specificity of MGLP and further improvement of the thermostability using protein engineering based on the results of this study. In addition, the three-dimensional structure of the enzyme must be determined to elucidate the relationship between its structure and unique substrate specificity.

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REFERENCES

1. Chahinian, H., Vanot, G., Ibrik, A., Rugani, N., Sarda, L., and Comeau, L.C. (2000) Production of extracellular lipases by *Penicillium cyclopium* Purification and characterization of a partial

acylglycerol lipase. *Biosci. Biotechnol. Biochem.* **64**, 215–222
2. Moriyama, T., Urade, R., and Kito, M. (1999) Purification and characterization of diacylglycerol lipase from human platelets. *J. Biochem.* **125**, 1077–1085
3. Lee, M.W. and Severson, D.L. (1994) Partial purification of a diacylglycerol lipase from bovine aorta. *Biochem. J.* **298**, 213–219
4. Benkirane, M., Meignen, J.M., Boyer, J., and Verine, A. (1989) Lipoprotein lipase in rat heart—I. Characterization of tri-, di- and monoacylglycerol lipase activities in post-heparin effluents. *Comp. Biochem. Physiol. [B]* **94**, 13–18
5. Severson, D.L. and Hee-Cheong, M. (1986) Diacylglycerol lipase and kinase activities in rabbit aorta and coronary microvessels. *Biochem. Cell Biol.* **64**, 976–983
6. Stam, H., Broekhoven-Schokker, S., and Hülsmann, W.C. (1986) Characterization of mono-, di- and triacylglycerol lipase activities in the isolated rat heart. *Biochim. Biophys. Acta* **875**, 76–86
7. Keough, D.T., de Jersey, J., and Zerner, B. (1985) The relationship between the carboxylesterase and monoacylglycerol lipase activities of chicken liver microsomes. *Biochim. Biophys. Acta* **829**, 164–172
8. Hee-Cheong, M., Fletcher, T., Kryski, S.K., and Severson, D.L. (1985) Diacylglycerol lipase and kinase activities in rat brain microvessels. *Biochim. Biophys. Acta* **833**, 59–68
9. Dejong, B.J.P., Kalkman, C., and Hülsmann, W.C. (1978) Partial purification and properties of monoacylglycerol lipase and two esterases isolated rat small intestinal epithelial cells. *Biochim. Biophys. Acta* **530**, 56–66
10. Tornqvist, H. and Belfrage, P. (1976) Purification and some properties of a monoacylglycerol-hydrolyzing enzyme of rat adipose tissue. *J. Biol. Chem.* **251**, 813–819
11. Somma-Delpéro, C., Valette, A., Lepetit-Thévenin, J., Nobili, O., Boyer, J., and Vérine, A. (1995) Purification and properties of a monoacylglycerol lipase in human erythrocytes. *Biochem. J.* **312**, 519–525
12. Toida, J., Kondoh, K., Fukuzawa, M., Ohnishi, K., and Sekiguchi, J. (1995) Purification and characterization of a lipase from *Aspergillus orizae*. *Biosci. Biotech. Biochem.* **59**, 1199–1203
13. Yamaguchi, S. and Mase, T. (1991) Purification and characterization of mono- and diacylglycerol lipase isolated from *Penicillium camembertii* U-150. *Appl. Microbiol. Biotechnol.* **34**, 720–725
14. Farooqui, A.A., Taylor, W.A., and Horrocks, L.A. (1984) Separation of bovine brain mono- and diacylglycerol lipases by heparin sepharose affinity chromatography. *Biochem. Biophys. Res. Commun.* **122**, 1241–1246
15. Yamaguchi, S., Takeuchi, K., Mase, T., and Matsuura, A. (1997) Efficient expression of mono- and diacylglycerol lipase gene from *Penicillium camembertii* U-150 in *Aspergillus oryzae* under the control of its own promoter. *Biosci. Biotechnol. Biochem.* **61**, 800–805
16. Imamura, S. and Kitaura, S. (2000) Purification and characterization of a monoacylglycerol lipase from the moderately thermophilic *Bacillus* sp. H-257. *J. Biochem.* **127**, 419–425
17. Chang, A.C.Y. and Cohen, S.N. (1978) Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. *J. Bacteriol.* **134**, 1141–1156
18. Vieira, J. and Messing, J. (1987) Production of single-stranded plasmid DNA in *Methods in Enzymology* (Abelson, J.N. and Simon, M.I., eds.) Vol. 153, pp. 3–11, Academic Press, New York
19. Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., and Erlich, H.A. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487–491
20. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. Saito, H. and Miura, K. (1963) Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim. Biophys. Acta* **72**, 619–629
22. Davies, R.W., Botstein, D., and Roth, J.R. (1980) *A Manual for Genetic Engineering: Advanced Bacterial Genetics*, pp. 159–167, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
23. Mizusawa, S., Nishimura, S., and Seela, F. (1986) Improvement of the dideoxy chain termination method of DNA sequencing by use of deoxy-7-deazaguanosine triphosphate in place of dGTP. *Nucleic Acids Res.* **14**, 1319–1324
24. Tsuchiya, A., Nakazawa, H., Toida, J., Ohnishi, K., and Sekiguchi, J. (1996) Cloning and nucleotide sequence of the mono- and diacylglycerol lipase gene (*mdlB*) of *Aspergillus orizae*. *FEMS Microbiol. Lett.* **143**, 63–67
25. Yamaguchi, S., Mase, T., and Takeuchi, K. (1991) Cloning and structure of the mono- and diacylglycerol lipase-encoding gene from *Penicillium camembertii* U-150. *Gene* **103**, 61–67
26. Redenbach, M., Kieser, H.M., Denapaite, D., Eichner, A., Cullum, J., Kinashi, H., and Hopwood, D.A. (1996) A set of ordered cosmids and a detailed genetic and physical map for the 8 Mb *Streptomyces coelicolor* A3(2) chromosome. *Mol. Microbiol.* **21**, 77–96
27. Kugimiya, W., Otani, Y., and Hashimoto, Y. (1992) Molecular cloning and structure of the gene for esterase from a thermophilic bacterium, *Bacillus stearothermophilus* IFO 12550. *Biosci. Biotechnol. Biochem.* **56**, 2074–2075
28. Jaeger, K.E., Ransac, S., Dijkstra, B.W., Colson, C., Heuvel, M., and Misset, O. (1994) Bacterial lipases. *FEMS Microbiol. Rev.* **15**, 29–63
29. Kim, H.K., Park, S.Y., Lee, J.K., and Oh, T.K. (1998) Gene cloning and characterization of thermostable lipase from *Bacillus stearothermophilus* L1. *Biosci. Biotechnol. Biochem.* **62**, 66–71
30. Rúa, M.L., Atomi, H., Schmidt-Dannert, C., and Schmid, R.D. (1998) High-level expression of the thermoalkalophilic lipase from *Bacillus thermocatenulatus* in *Escherichia coli*. *Appl. Microbiol. Biotechnol.* **49**, 405–410