



TITLE OF THE INVENTION

A gene coding a protein having a lipase activity
and a process for producing the same

BACKGROUND OF THE INVENTION

5 1. Field of the Invention

This invention relates to a gene coding a protein having a lipase activity originated from a bacterium of Pseudomonas genus, a recombinant DNA containing the gene, a bacterium of Pseudomonas genus containing the DNA and
10 a process for producing a protein having a lipase activity, characterized by secretory production outside bacterial cells.

2. Description of the Related Art

Lipases have been used as a lipid-hydrolyzing
15 enzyme, for oil and fat processing, clinical diagnostic medicine, detergent, digestant, etc., and further, in recent years, lipases have been an important enzyme for catalyzing ester hydrolysis, ester synthesis or transesterification, as processes for producing chemical products,
20 particularly, optically active compounds. Further, it has been known that bacteria of Pseudomonas genus produce a lipase useful as a catalyst for ester hydrolysis, ester synthesis or transesterification(see Japanese patent application laid-open No. Sho 62-166898).



In order to commercially utilize a lipase originated from bacterium of *Pseudomonas* genus and provided with such characteristics, production of an enzyme in a large quantity has been desired. Further, for its production
5 on a commercial scale, secretory production of the enzyme into the culture solution has been regarded as effective in view of the advantages of continuous cultivation, easiness in the enzyme recovery, etc. In recent years, application of gene manipulation technique has been
10 introduced into production of useful proteins in a large quantity, and this has also been applied also to lipases of bacteria of *Pseudomonas* genus.

However, in spite of such attempts, an efficient secretory production system of lipases into the culture
15 solution has not yet been completed, and problems have been raised in respect of commercial utilization.

For example, when *Escherichia coli* is used as a host bacterium, recovery of the produced lipase has been carried out from the inside of the bacterial cells
20 (Japanese patent application laid-open Nos. Sho 62-188072 and Sho 62-228279 and Japanese patent application No. Hei 1-7757). In such a case of production inside the bacterial cells, the recovery of the produced substance requires a bacterium-breaking step such as
25 supersonic treatment and a number of purification steps



for removing impurities originated from host bacterium. Thus, such processes have been regarded as disadvantageous, in the aspect of production of such a substance.

Further, when *Pseudomonas* bacterium is used as
5 a host bacterium, recovery of lipase has been carried out from the inside of the culture solution which is advantageous for commercial utilization (Japanese patent application Nos. Sho 63-300386 and Hei 1-338250). However, in such cases, cultivation has been carried out e.g. for
10 3 days for the production. Thus, such production cannot always be regarded as efficient.

In solving the above-mentioned problems, the present inventors considered that when a strain producing a protein having a lipase activity originated from
15 *Pseudomonas* genus bacterium, in a high extent secretion, is created according to a gene recombination technique, it might be possible to produce the lipase on a commercial scale, and have made extensive research. As a result, we have found that when a DNA fragment containing a gene
20 coding a protein having a lipase activity is isolated from a chromosome DNA of *Pseudomonas* genus bacterium, to obtain a recombinant DNA having the DNA fragment, then *Pseudomonas* genus bacterium transformed by the recombinant DNA secretorily produces a notably large
25 quantity of a protein having a lipase activity in the culture solution, and have completed the present invention.



Namely, the object of the present invention is to provide a DNA fragment containing a gene coding a protein having a lipase activity originated from a bacterium of *Pseudomonas* genus, a recombinant DNA
5 having the above DNA fragment, a bacterium of *Pseudomonas* genus suitable to efficient secretory production of a protein transformed by the above recombinant DNA and having a lipase activity, and a process for producing a protein having a lipase activity, using the above
10 bacterium.

SUMMARY OF THE INVENTION

The present invention has the following constitutions:

- (1) A gene coding a protein having a lipase activity originated from a bacterium of *Pseudomonas* genus, characterized
15 by having the following base sequence:

10	20	30	40	50	60
5' ATGAAACATT	TAATACAGTT	TGTTTTGCTA	ATGTCTGCGC	CCCAAACAAC	AAAAACAGGT
70	80	90	100	110	120
CATCACATGC	GCAGGCTCTC	CCTACTCATC	CCTTGGCGGG	GATGCATCCT	GTCCATCGTC
130	140	150	160	170	180
AGTGAACGTG	CCATAGCAGC	ACCCTCGCCG	TATTCCACCA	TGATCGTGTT	CGGCGACAGC
190	200	210	220	230	240
CTGAGCGACT	CGGGGCATTT	TCCAGGGGTC	GGCACAGGCA	TGCGCTTCAC	CAACCGCACC
250	260	270	280	290	300
GGGCCGACGT	ACAGGGATTA	CCGGGGGGAG	GAATATGCTG	CAGTAACGCC	CACGGGGCTG
310	320	330	340	350	360
GGCACCCAAC	TGGGGATCGC	CCCCGCAGAC	CTGCGCCCGT	CCACTTCGCC	GGGCAACTCT
370	380	390	400	410	420



CTTGCAGGGG	AGCCCGACGG	CAACAACCTGG	GCGGTCGGCG	GCTATCGCAC	AGACCAGATC
430	440	450	460	470	480
CTCAATTCCA	TCAAGACCGA	GTCAAAGGTC	GCCATCCCGG	ATGACTGGTC	CTTCGTCGGC
490	500	510	520	530	540
GGCTACGTCC	TGCGCAGCAA	GCCTGGGTAC	CTTGTGCAGA	ACAGCTTCAC	GGCTGACCCC
550	560	570	580	590	600
AAGGCGTTGT	ATTTCAATTC	AGGCGGCGGT	AATGACTTTC	TCCAAGGCAA	GGTCACCAAC
610	620	630	640	650	660
CCCGCCGAGG	CAGGCCAAGC	CGCACAAAGG	CTGGCGGCAA	GCGCCACAC	CCTGCAACAG
670	680	690	700	710	720
GCAGGGGCAC	GCTACATCAT	GGTCTGGCTG	CTGCCCCGATC	TGGGCCTGAC	GCCAGCCGTT
730	740	750	760	770	780
TACGGTACAC	CCACCCAGGC	TGGCACCAGC	GCCCTGAGCG	CCCTGTTCAA	TCATGAGCTA
790	800	810	820	830	840
ACCCAGCAAC	TGGCGCAGAT	AGACGCCGAA	GTGATTCCCC	TCAATATCCC	GCTGCTGCTG
850	860	870	880	890	900
CGTGAAGCCA	TCGCCGACCC	CGCCCGTTAT	GGCCTGGCAC	TGGGGCAGGA	CCTTGTGCGG
910	920	930	940	950	960
ACCTGTTTCA	ATGGCGAAGA	GTGTACGGAA	AACCCGCACT	ACGGACTCAA	CAGCGCAACC
970	980	990	1000	1010	1020
CCCAACCCGG	CCAAACTGCT	ATTCAACGAC	TCGGTACACC	CCACCGAGAC	AGGGCAACAG
1030	1040	1050	1060	1070	1080
CTGATTGCCG	ACTATGCCTA	CTCATTGCTG	GCCGTGCCGT	GGGAGCTGAC	CTTGTTACCC
1090	1100	1110	1120	1130	1140
GTTATGGCCC	AAGGCTCGCT	CAACGCCCAT	CAAGACCAGT	TGCGCAACCA	ATGGGCTGGC
1150	1160	1170	1180	1190	1200
GACGACGGTC	AATGGCAAGC	CATTGGCCAG	TGGCGAACCC	TGCTCGCCGG	TGGCGGGCAA
1210	1220	1230	1240	1250	1260
CGCCTGAAAA	TCGACAAGCA	GACCACGGCC	GTGAAGGCAG	ATGGCAAAGG	CTATAACCTC



1270	1280	1290	1300	1310	1320
AATATCGGCA	CCAGCTACAG	GCTGGATGAC	AACTGGCGAT	TTGGCATTGC	CGGCGGGTTT
1330	1340	1350	1360	1370	1380
TACCGGCAAC	GCCTGGAAAC	CGGGGCCAAT	AAATCGGACT	ACAAGCTCAA	CAGCTATCTG
1390	1400	1410	1420	1430	1440
GGTAGCGTGT	TTGCCCAATA	CCAGCACAAT	CACTGGTGGG	GGGATGCGGC	ACTGACGTTG
1450	1460	1470	1480	1490	1500
GGCAGGCTGG	ATTACGACAG	CCTGAAACGA	AAATTCGCGC	TTGGGGTGGG	TAGTGATATG
1510	1520	1530	1540	1550	1560
GAGCAAGGCC	AGGCTGACGG	CCATTTGCGA	GCATTGGTAC	ACGCCTGGGT	TATGAAATCG
1570	1580	1590	1600	1610	1620
CACAGGCATC	TGACCTGTGG	CAGCTCTCCC	CCTTTATCAG	CGCCGTTACT	CGCGGGTGGA

AGTTAA 3'

(2) A gene according to item (1) wherein said bacterium of *Pseudomonas* genus is *Pseudomonas fragi* IFO 120 49 strain.

(3) A recombinant DNA which is replicative with a bacterium of *Pseudomonas* genus having said gene coding a protein having a lipase activity identified in item (1), incorporated into a broad host range vector of Gram-negative bacteria.

(4) A recombinant DNA which is replicative with a bacterium of *Pseudomonas* genus having said gene coding a protein having a lipase activity identified in item (2), incorporated into a broad host range vector of Gram-negative bacteria.



(5) A bacterium of Pseudomonas genus transformed by a recombinant DNA according to item (3).

(6) A bacterium of Pseudomonas genus transformed by a recombinant DNA which is replicative with a bacterium
5 of Pseudomonas genus having said gene coding a protein having a lipase activity identified in item (1), incorporated into a broad host range vector of Gram-negative bacteria.

(7) A process for producing a protein having a lipase
10 activity, which process comprises cultivating a bacterium of Pseudomonas genus transformed by a recombinant DNA according to item (3), to thereby produce a protein having a lipase activity in the culture medium, and recovering this protein from the culture medium.

15 BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows an example of a restriction enzyme map of Eco RI 3.7 Kb fragment having a plasmid pCCB inserted thereinto and the results of a deletion experiment wherein the presence or absence of clear zone formation is made
20 an indication. The symbol (+) shows a clear zone formation.

Fig. 2 shows a construction figure of the expression plasmid pUC-RSFKmCCB of a protein having a lipase activity.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

25 The present invention will be described in more detail.



① Cloning of a gene coding a protein having a lipase activity:

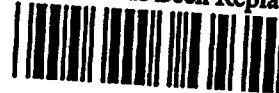
The chromosome DNA of *Pseudomonas fragi* IFO 12049 strain can be prepared for example according to Smith's method (Smith, M.G., Methods in Enzymology, Academic press, New York, 12, part A, p. 545, (1967)).

Incorporation of a chromosome DNA into a vector DNA can be carried out by digesting the chromosome DNA and the vector DNA with a restriction enzyme, followed by mixing the two and treating the mixture with a DNA lipase.

Examples of the restriction enzyme are EcoRI, BamHI, Sau3AI, etc. As the vector DNA, known ones such as pUC9, λ gt10, Charomid DNA, etc. are utilizable. The thus obtained recombinant DNA can transform *Escherichia coli* according to a known method such as CaCl_2 method, in vitro packaging method, etc.

Choice of a transformed strain having a lipase activity is carried out by isolating a strain forming a clear zone accompanying the hydrolysis of tributyrin on a tributyrin-agar medium. A recombinant DNA containing a gene coding a protein having a lipase activity can be obtained from the above obtained transformed strain, according to a known technique such as alkali method.

Next, the region necessary for the expression of the protein having a lipase activity in the transformed bacterium can be determined by subcloning the above



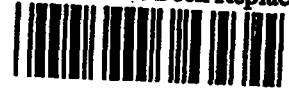
recombinant DNA using various restriction enzymes, and observing the clear zone-formability of such transformed strain on the tributyrin-agar medium. Further, the gene coding the protein having a lipase activity and the base
5 sequence in the region necessary for the expression can be determined according to a known technique such as dioxymethoxy method (Sanger.F., et al, Proc. Natl. Acad. Sci. U.S.A., 74, 5463 (1977)).

② Production of the protein having a lipase activity:

10 When a DNA fragment containing a gene coding the protein obtained in the above manner is introduced into a plasmid vector which is replicative inside the bacterium of Pseudomonas genus, according to a known conjunction-transmission method or the like, it is
15 possible to construct a recombinant DNA for lipase expression.

As such a plasmid vector, for example, a broad range host vector RSF1010, those obtained by introducing a suitable selective marker such as kanamycin-resistant
20 gene into RSF1010, etc. are usable.

Further, by cultivating a bacterium of Pseudomonas genus transformed by the recombinant DNA obtained in the above manner, under optimum conditions for enzyme production, a protein having a lipase activity is
25 produced. Further, by measuring the lipase activity in the culture solution, it is possible to obtain an optimum



host bacterium of *Pseudomonas* genus for secretorily expressing a protein having a lipase activity. As the thus obtained bacterium of *Pseudomonas* genus, for example, *Pseudomonas putida* AC10 strain and the like are usable.

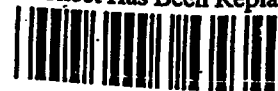
5 Further, the measurement of lipase activity can be carried out using a commercially available, lipase activity-measuring kit, or the like. *Pseudomonas putida* AC10 strain having a recombinant DNA obtained according to the present invention introduced therein is cultivated
10 under optimum conditions for enzyme production, and a protein having a lipase activity is separated from the culture medium and purified.

The present invention will be described in more detail by way of Example.

15 Example

(1) Preparation of chromosome DNA:

Pseudomonas fragi IFO 12049 strain was cultivated in an LB medium (1% bactotripton, 0.5% yeast extract and 0.5% NaCl), at 30°C overnight. The bacterial cells were
20 collected, followed by washing and suspending the cells in a solution of 50 mM Tris-HCl buffer (pH 8.0) and 10 mM EDTA (12 ml). Lysozyme (1 mg) was added to the resulting suspension, followed by allowing the mixture to stand at room temperature for one hour,
25 adding to the resulting solution, Protienase K (trademark of product made by Merck Co., Ltd.) so as to give



a concentration of 100 µg/ml, in the presence of 0.5% SDS, slowly shaking the mixture at 55°C for 3 hours, extracting this resulting sample with phenol and precipitating it with ethanol to prepare chromosome DNA.

- 5 (2) Cloning of a gene coding a protein having a lipase activity and analysis of the gene:

The thus prepared chromosome DNA was digested with a restriction enzyme EcoRI, followed by subjecting the resulting substance to electrophoresis in an agarose gel
10 to recover fragments of 2 to 4 Kb, linking the recovered DNA fragment to EcoRI site of Charomid 9-42 (trademark of product made by Nippon Gene Co., Ltd.) with a DNA lipase, and infecting the linked DNA in the form of phage with Escherichia coli VCS 257 using λDNA in vitro packaging
15 kit (Gigapack IIPLUS (tradename of product made by STRATAGENE Co., Ltd.)) to effect transduction.

The resulting transformed strain was subjected to plating on an LB medium containing ampicillin (50 µg/ml) and 1% tributyrin, at 37°C for 24 hours to obtain two
20 clear zone-forming strains from among about 1,100 transformed strains, extracting a plasmid from the above two clear zone-forming strains, digesting EcoRI, analyzing inserted DNA fragments by agarose gel electrophoresis to confirm that both the strains contained about 3.7 Kb EcoRI
25 fragment, and subcloning the 3.7 Kb EcoRI DNA fragment



- 12 -

at the EcoRI site of pTV 119 N (made by Takara Shuzo Co., Ltd.), to prepare a plasmid pCCB. Fig. 1 shows a restriction enzyme map of 3.7 Kb DNA fragments having EcoRI inserted at pCCB. Next, the 3.7 Kb EcoRI fragments
5 of the plasmid pCCB were broken by various kinds of restriction enzymes, followed by subcloning the respective fragments at pTV 119N to transform Escherichia coli MV 1184 strain, and observing the presence or absence of clear zone formation on the tributyrin-containing LB
10 medium, in the same manner as above.

As a result, a clear zone formation was observed in Escherichia coli having a plasmid pCCB-XS having EcoRI-XhoI 2.1 Kb fragment, as shown in Fig. 1. As a result, it was suggested that a gene coding a protein
15 having a lipase activity and a region necessary for its expression were present in EcoRI-XhoI 2.1 Kb fragment.

(3) Determination of base sequence:

The base sequence of inserted EcoRI 3.7 Kb DNA fragment of the plasmid pCCB was determined according
20 to dideoxy method (F. Sanger et al, Proc. Natl. Acad. Sci. U.S.A., 74, 5463 (1977)). As a result, an open reading frame was present within a region shown by an arrow mark (→) in Fig. 1.

The base sequence of the gene and the corresponding
25 amino acid sequence table are shown after the end of
DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS.



(4) Production of a protein having a lipase activity:

An expression plasmid pUC-RSFKmCCB was constructed according to the steps shown in Fig. 2.

To 5.9 Kb fragment obtained by digesting PstI and
5 PvuII of a broad range host vector RSF1010 was linked
2.7 Kb fragment obtained by digesting PstI and SmaI of
pUC19 to obtain a plasmid pUC-RSF, followed by introduc-
ing into PstI site of this plasmid, 1.3 Kb fragment of
PstI containing a kanamycin-resistant gene, and
10 introducing into the EcoRI site of the resulting plasmid,
pUC-RSFKm, EcoRI 3.7 Kb fragment of pCCB, to construct
the expression plasmid pUC-RSFKmCCB.

This plasmid pUC-RSFKmCCB was introduced into
Pseudomonas putida AC10 strain, according to Simon et al's
15 conjugation method (R. Simon et al, Biotechnology,
1.784 (1983)). This Pseudomonas putida AC10 (pUC-RSFKmCCB)
strain was cultivated on an LB medium containing ampicillin
and kanamycin (each, 50 µg/ml), at 30°C overnight. The
lipase activity of the filtrate of the culture medium
20 was measured using Lipase Kit S (tradename of product
made by Dainippon Pharmaceutical Co., Ltd.) to give
268 BALB unit per 100 µl of the filtrate.

The results of measurements of Pseudomonas fragi
IFO 12049 strain and Pseudomonas putida AC 10 strain,
25 carried out under the same conditions were both, values
lower than the lower limit of the measurement sensitivity



(50 BALB unit) per 100 μ l of the filtrate. In addition, the measurement method and the calculation method of measurement unit were carried out according to the method of Lipase Kit S.

5 In the above Example, plasmid preparation from bacterial cells, the reaction using restriction enzymes, etc. and treatment of DNA fragment were carried out in a conventional manner unless otherwise indicated. In addition, as to the restriction enzymes, DNA ligase,
10 etc., those prepared by Takara Shuzo Co., Ltd. and Japan Gene Co., Ltd. were used.

In addition, the sequence listing in the present invention is as follows:

Sequence listing

15 sequence No.: 1
Length of sequence: 1,626
Type of sequence: nucleic acid
Number of chain: two
Topology: linear chain
20 Kind of sequence: Genomic DNA
Hypothetical: Yes
Origin
Name of living: *Pseudomonas fragi*
Name of strain: IFO 12049



ATG AAA CAT TTA ATA CAG TTT GTT TTG CTA ATG TCT GCG CCC CAA ACA
Met Lys His Leu Ile Gln Phe Val Leu Leu Met Ser Ala Pro Gln Thr

ACA AAA ACA GGT CAT CAC ATG CGC AGG CTC TCC CTA CTC ATC CCT TGG
Thr Lys Thr Gly His His Met Arg Arg Leu Ser Leu Leu Ile Pro Trp

CGG GGA TGC ATC CTG TCC ATC GTC AGT GAA CGT GCC ATA GCA GCA CCC
Arg Gly Cys Ile Leu Ser Ile Val Ser Glu Arg Ala Ile Ala Ala Pro

TCG CCG TAT TCC ACC ATG ATC GTG TTC GGC GAC AGC CTG AGC GAC TCG
Ser Pro Tyr Ser Thr Met Ile Val Phe Gly Asp Ser Leu Ser Asp Ser

GGG CAT TTT CCA GGG GTC GGC ACA GGC ATG CGC TTC ACC AAC CGC ACC
Gly His Phe Pro Gly Val Gly Thr Gly Met Arg Phe Thr Asn Arg Thr

GGG CCG ACG TAC AGG GAT TAC CGG GGG GAG GAA TAT GCT GCA GTA ACG
Gly Pro Thr Tyr Arg Asp Tyr Arg Gly Glu Glu Tyr Ala Ala Val Thr

CCC ACG CGG CTG GGC ACC CAA CTG GGG ATC GCC CCC GCA GAC CTG CGC
Pro Thr Arg Leu Gly Thr Gln Leu Gly Ile Ala Pro Ala Asp Leu Arg

CCG TCC ACT TCG CCG GGC AAC TCT CTT GCA GGG GAG CCC GAC GGC AAC
Pro Ser Thr Ser Pro Gly Asn Ser Leu Ala Gly Glu Pro Asp Gly Asn

AAC TGG GCG GTC GGC GGC TAT CGC ACA GAC CAG ATC CTC AAT TCC ATC
Asn Trp Ala Val Gly Gly Tyr Arg Thr Asp Gln Ile Leu Asn Ser Ile

AAG ACC GAG TCA AAG GTC GCC ATC CCG GAT GAC TGG TCC TTC GTC GGC
Lys Thr Glu Ser Lys Val Ala Ile Pro Asp Asp Trp Ser Phe Val Gly

GGC TAC GTC CTG CGC AGC AAG CCT GGC TAC CTT GTG CAG AAC AGC TTC
Gly Tyr Val Leu Arg Ser Lys Pro Gly Tyr Leu Val Gln Asn Ser Phe

ACG GCT GAC CCC AAG GCG TTG TAT TTC ATT TCA GGC GGC GGT AAT GAC
Thr Ala Asp Pro Lys Ala Leu Tyr Phe Ile Ser Gly Gly Gly Asn Asp

TTT CTC CAA GGC AAG GTC ACC AAC CCC GCC GAG GCA GGC CAA GCC GCA
Phe Leu Gln Gly Lys Val Thr Asn Pro Ala Glu Ala Gly Gln Ala Ala

CAA AGG CTG GCG GCA AGC GCC CAC ACC CTG CAA CAG GCA GGG GCA CGC
Gln Arg Leu Ala Ala Ser Ala His Thr Leu Gln Gln Ala Gly Ala Arg

TAC ATC ATG GTC TGG CTG CTG CCC GAT CTG GGC CTG ACG CCA GCC GTT
Tyr Ile Met Val Trp Leu Leu Pro Asp Leu Gly Leu Thr Pro Ala Val

TAC GGT ACA CCC ACC CAG GCT GGC ACC AGC GCC CTG AGC GCC CTG TTC
Tyr Gly Thr Pro Thr Gln Ala Gly Thr Ser Ala Leu Ser Ala Leu Phe

AAT CAT GAG CTA ACC CAG CAA CTG GCG CAG ATA GAC GCC GAA GTG ATT
Asn His Glu Leu Thr Gln Gln Leu Ala Gln Ile Asp Ala Glu Val Ile

CCC CTC AAT ATC CCG CTG CTG CTG CGT GAA GCC ATC GCC GAC CCC GCC
Pro Leu Asn Ile Pro Leu Leu Leu Arg Glu Ala Ile Ala Asp Pro Ala

CGT TAT GGC CTG GCA CTG GGG CAG GAC CTT GTC GCG ACC TGT TTC AAT
Arg Tyr Gly Leu Ala Leu Gly Gln Asp Leu Val Ala Thr Cys Phe Asn

GGC GAA GAG TGT ACG GAA AAC CCG CAG TAC GGA CTC AAC AGC GCA ACC



Gly Glu Glu Cys Thr Glu Asn Pro Gln Tyr Gly Leu Asn Ser Ala Thr

CCC AAC CCG GCC AAA CTG CTA TTC AAC GAC TCG GTA CAC CCC ACC GAG
Pro Asn Pro Ala Lys Leu Leu Phe Asn Asp Ser Val His Pro Thr Glu

ACA GGG CAA CAG CTG ATT GCC GAC TAT GCC TAC TCA TTG CTG GCC GTG
Thr Gly Gln Gln Leu Ile Ala Asp Tyr Ala Tyr Ser Leu Leu Ala Val

CCG TGG GAG CTG ACC TTG TTA CCC GTT ATG GCC CAA GGC TCG CTC AAC
Pro Trp Glu Leu Thr Leu Leu Pro Val Met Ala Gln Gly Ser Leu Asn

GCC CAT CAA GAC CAG TTG CGC AAC CAA TGG GCT GGC GAC GAC GGT CAA
Ala His Gln Asp Gln Leu Arg Asn Gln Trp Ala Gly Asp Asp Gly Gln

TGG CAA GCC ATT GGC CAG TGG CGA ACC CTG CTC GCC GGT GGC GGG CAA
Trp Gln Ala Ile Gly Gln Trp Arg Thr Leu Leu Ala Gly Gly Gly Gln

CGC CTG GAA ATC GAC AAG CAG ACC ACG GCC GTG AAG GCA GAT GGC AAA
Arg Leu Glu Ile Asp Lys Gln Thr Thr Ala Val Lys Ala Asp Gly Lys

GGC TAT AAC CTC AAT ATC GGC ACC AGC TAC AGG CTG GAT GAC AAC TGG
Gly Tyr Asn Leu Asn Ile Gly Thr Ser Tyr Arg Leu Asp Asp Asn Trp

CGA TTT GGC ATT GCC GGC GGG TTT TAC CGG CAA CGC CTG GAA ACC GGG
Arg Phe Gly Ile Ala Gly Gly Phe Tyr Arg Gln Arg Leu Glu Thr Gly

GCC AAT AAA TCG GAC TAC AAG CTC AAC AGC TAT CTG GGT AGC GTG TTT
Ala Asn Lys Ser Asp Tyr Lys Leu Asn Ser Tyr Leu Gly Ser Val Phe



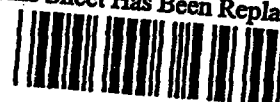
GCC CAA TAC CAG CAC AAT CAC TGG TGG GGG GAT GCG GCA CTG ACG TTG
Ala Gln Tyr Gln His Asn His Trp Trp Gly Asp Ala Ala Leu Thr Leu

GGC AGG CTG GAT TAC GAC AGC CTG AAA CGA AAA TTC GCG CTT GGG GTG
Gly Arg Leu Asp Tyr Asp Ser Leu Lys Arg Lys Phe Ala Leu Gly Val

GGT AGT GAT ATG GAG CAA GGC CAG GCT GAC GGC CAT TTG CGA GCA TTG
Gly Ser Asp Met Glu Gln Gly Gln Ala Asp Gly His Leu Arg Ala Leu

GTA CAC GCC TGG GTT ATG AAA TCG CAC AGG CAT CTG ACC TGT GGC AGC
Val His Ala Trp Val Met Lys Ser His Arg His Leu Thr Cys Gly Ser

TCT CCC CCT TTA TCA GCG CCG TTA CTC GCG GGT GGA AGT TAA
Ser Pro Pro Leu Ser Ala Pro Leu Leu Ala Gly Gly Ser ***



WHAT WE CLAIM IS:

1. A gene coding a protein having a lipase activity originated from a bacterium of Pseudomonas genus, characterized by having the following base sequence:

10	20	30	40	50	60
5'ATGAAACATT	TAATACAGTT	TGTTTTGCTA	ATGTCTGCGC	CCCAAACAAC	AAAAACAGGT
70	80	90	100	110	120
CATCACATGC	GCAGGCTCTC	CCTACTCATC	CCTTGGCGGG	GATGCATCCT	GTCCATCGTC
130	140	150	160	170	180
AGTGAACGTG	CCATAGCAGC	ACCCTCGCCG	TATTCCACCA	TGATCGTGTT	CGGCGACAGC
190	200	210	220	230	240
CTGAGCGACT	CGGGGCATTT	TCCAGGGGTC	GGCACAGGCA	TGCGCTTCAC	CAACCGCACC
250	260	270	280	290	300
GGGCCGACGT	ACAGGGATTA	CCGGGGGGAG	GAATATGCTG	CAGTAACGCC	CACGCGGCTG
310	320	330	340	350	360
GGCACCCAAC	TGGGGATCGC	CCCCGCAGAC	CTGCGCCCGT	CCACTTCGCC	GGGCAACTCT
370	380	390	400	410	420
CTTGCAGGGG	AGCCCCGACGG	CAACAACTGG	GCGGTCGGCG	GCTATCGCAC	AGACCAGATC
430	440	450	460	470	480
CTCAATTCCA	TCAAGACCGA	GTCAAAGGTC	GCCATCCCGG	ATGACTGGTC	CTTCGTGGGC
490	500	510	520	530	540
GGCTACGTCC	TGCGCAGCAA	GCCTGGCTAC	CTTGTGCAGA	ACAGCTTCAC	GGCTGACCCC
550	560	570	580	590	600
AAGGCGTTGT	ATTTCAATTC	AGGCGGCGGT	AATGACTTTC	TCCAAGGCAA	GGTCACCAAC
610	620	630	640	650	660
CCCGCCGAGG	CAGGCCAAGC	CGCACAAAGG	CTGGCGGCAA	GCGCCCACAC	CCTGCAACAG
670	680	690	700	710	720
GCAGGGGCAC	GCTACATCAT	GGTCTGGCTG	CTGCCCGATC	TGGGCCTGAC	GCCAGCCGTT
730	740	750	760	770	780
TACGGTACAC	CCACCCAGGC	TGGCACCAGC	GCCCTGAGCG	CCCTGTTCAA	TCATGAGCTA

This Sheet Has Been Replaced



- 20 -



790	800	810	820	830	840
ACCCAGCAAC	TGGCGCAGAT	AGACGCCGAA	GTGATTCCCC	TCAATATCCC	GCTGCTGCTG
850	860	870	880	890	900
CGTGAAGCCA	TCGCCGACCC	CGCCCGTTAT	GGCCTGGCAC	TGGGGCAGGA	CCTTGTCGCG
910	920	930	940	950	960
ACCTGTTTCA	ATGGCGAAGA	GTGTACGGAA	AACCCGCAGT	ACGGA CTCAA	CAGCGCAACC
970	980	990	1000	1010	1020
CCCAACCCGG	CCAAACTGCT	ATTCAACGAC	TCGGTACACC	GCACCGAGAC	AGGGCAACAG
1030	1040	1050	1060	1070	1080
CTGATTGCCG	ACTATGCCTA	CTCATTGCTG	GCCGTGCCGT	GGGAGCTGAC	CTTGTTACCC
1090	1100	1110	1120	1130	1140
GTTATGGCCC	AAGGCTCGCT	CAACGCCCAT	CAAGACCAGT	TGCGCAACCA	ATGGGCTGGC
1150	1160	1170	1180	1190	1200
GACGACGGTC	AATGGCAAGC	CATTGGCCAG	TGGCGAACCC	TGCTCGCCGG	TGGCGGGCAA
1210	1220	1230	1240	1250	1260
CGCCTGGAAG	TCGACAAGCA	GACCACGGCC	GTGAAGGCAG	ATGGCAAAGG	CTATAACCTC
1270	1280	1290	1300	1310	1320
AATATCGGCA	CCAGCTACAG	GCTGGATGAC	AACTGGCGAT	TTGGCATTGC	CGGCGGGTTT
1330	1340	1350	1360	1370	1380
TACCGGCAAC	GCCTGGAAAC	CGGGGCCAAT	AAATCGGACT	ACAAGCTCAA	CAGCTATCTG
1390	1400	1410	1420	1430	1440
GGTAGCGTGT	TTGCCCAATA	CCAGCACAAAT	CACTGGTGGG	GGGATGCGGC	ACTGACGTTG
1450	1460	1470	1480	1490	1500
GGCAGGCTGG	ATTACGACAG	CCTGAAACGA	AAATTCGCGC	TTGGGGTGGG	TAGTGATATG
1510	1520	1530	1540	1550	1560
GAGCAAGGCC	AGGCTGACGG	CCATTTGCGA	GCATTGGTAC	ACGCCTGGGT	TATGAAATCG
1570	1580	1590	1600	1610	1620
CACAGGCATC	TGACCTGTGG	CAGCTCTCCC	CCTTTATCAG	CGCCGTTACT	CGCGGGTGGA
AGTTAA 3'					



2. A gene according to claim 1 wherein said bacterium of *Pseudomonas* genus is *Pseudomonas fragi* IFO 120 49 strain.

3. A recombinant DNA which is replicative with a bacterium of *Pseudomonas* genus having said gene coding a protein having a lipase activity identified in claim 1, incorporated into a broad host range vector of Gram-negative bacteria.

4. A recombinant DNA which is replicative with a bacterium of *Pseudomonas* genus having said gene coding a protein having a lipase activity identified in claim 2, incorporated into a broad host range vector of Gram-negative bacteria.

5. A bacterium of *Pseudomonas* genus transformed by a recombinant DNA according to claim 3.

6. A bacterium of *Pseudomonas* genus transformed by a recombinant DNA which is replicative with a bacterium of *Pseudomonas* genus having said gene coding a protein having a lipase activity identified in claim 1, incorporated into a broad host range vector of Gram-negative bacteria.

7. A process for producing a protein having a lipase activity, which process comprises cultivating a bacterium of *Pseudomonas* genus transformed by a recombinant DNA according to claim 3, to thereby produce a protein having a lipase activity in the culture medium, and recovering this protein from the culture medium.