microorganisms-origin esterases had problems such that they were disadvantageous in activity, stability, or specificity, or that the esterase-producing microorganism had no high productivity. Moreover, even by using a gene recombinant microorganism, it is not necessarily easy to increase the esterase productivity thereof to the level applicable for industrial production merely by magnifying the esterase gene. Besides, even though the productivity of esterase is increased, the extracellular secretion of esterase is occasionally insufficient, and thereby complicated procedure is required for recovery of the enzyme.

As a result of various investigations, the present inventors have succeeded in obtaining a gene (DNA) coding for a polypeptide which participates in the mechanism of secretion of esterase by a microorganism of the genus *Serratia* and further have found that the esterase-producing microorganisms can show remarkably increased capability of secretion of esterase by transforming said microorganism with a recombinant plasmid containing said DNA. The present invention has been accomplished based on these new findings.

Summary Description of the Invention

An object of the present invention is to provide a gene coding for a polypeptide which participates in the mechanism of secretion of esterase produced by a microorganism of the genus *Serratia*. Another object of the invention is to provide a recombinant plasmid prepared by inserting said gene into a vector plasmid and further a transformant

containing said recombinant plasmid. A further object of the invention is to provide a method for the production of an esterase by cultivating said transformant and collecting the esterase accumulated outside and inside the cells of the transformant.

Brief Description of Drawing

Fig. 1 shows restriction endonuclease maps of each of plasmid DNAs pPKHE200, pPKHE75, pPKHE35, pPKHE90 and pPKHE65 which were isolated from the recombinant cells in Example 2.

Detailed Description of the Invention

The gene encoding a polypeptide which participates in the mechanism of secretion of esterase (hereinafter, referred to as "esterase secretory gene") is a double stranded DNA of 4466 base pairs having three open reading frames (ORF) within the sequence, specifically DNA having the nucleotide sequence shown in SEQ ID NO:1 hereinafter.

The donor microorganisms for such an esterase secretory gene include any microorganisms belonging to the genus *Serratia* which has an esterase productivity, for example, *Serratia marcescens* Sr41 (FERM BP-487), *Serratia liquefaciens* ATCC 27592, *Serratia marcescens* ATCC 13880, *Serratia marcescens* ATCC 14764, *Serratia marcescens* ATCC 19180, *Serratia marcescens* ATCC 21074, *Serratia marcescens* ATCC 27117, *Serratia marcescens* ATCC 21212, etc.

The vector plasmid into which the esterase secretory gene is inserted includes any plasmids being replicable in

transformed cells. Among them, preferred example of said plasmid includes plasmids which have a copy number of 1 to several thousands and contain a resistant marker against antibiotics such as ampicillin, kanamycin, chloramphenicol, and further contain an appropriate promoter such as *lac*, *tac*, or *trp*. Moreover, the vector plasmids may further contain a plasmid-stabilizing gene such as *par* and *par*B.

These vector plasmids include, for example, pLG339 [Gene, Vol. 18, 332 (1982)], pBR322 [Gene, Vol. 2, 95 (1977)], pUC18 [Gene, Vol. 33, 103 (1985)], pUC19 [Gene, Vol. 33, 103 (1985)], pHSG298 [Gene, Vol. 61, 63 (1987)], pHSG299 [Gene, Vol. 61, 63 (1987)], and the like.

The above vector plasmids are commercially available or can be obtained from microbial cells containing these plasmids by a conventional method, for example, by "cleared lysate method" (cf. Yasuyuki Takagi, "Procedure for Experiment in Genetic Engineering", page 125, published by Kodansha, 1980), or by "alkaline lysis method" [cf. Maniatis et al., "Molecular Cloning", page 368, Cold Spring Harbor Laboratory, U.S.A. (1982)].

The host microorganisms (both for the recombination of plasmids and for the expression of the desired esterase), include any microorganisms which can be transformed with the recombinant plasmid and can replicate the plasmid therein, and can express the gene on the plasmid and can produce a functional protein. These host microorganisms include, for example, microorganisms belonging to the genus *Serratia* or the

genus *Escherichia*, specifically *Serratia marcescens* Sr41 and various mutant strains derived therefrom, for example, *Serratia marcescens* M-1 (FERM BP-4068), *Serratia marcescens* TT392 [cf. Journal of Bacteriology, Vol. 161, 1 (1985)], or *Escherichia coli* K12 DH5 [cf. Maniatis et al., "Molecular Cloning", 2nd volume, A10, Cold Spring Harbor Laboratory, U.S.A. (1989)].

In addition, there may be used as the host microorganisms any other microorganisms containing a recombinant plasmid to which an esterase gene of the genus *Serratia* is inserted, for example, *Serratia marcescens* TA5025 (FERM BP-4067).

The chromosomal DNA containing the esterase secretory gene can easily be obtained from microorganisms containing said gene by a conventional method, for example, by treating the microbial cells with a lysozyme and further with a surfactant (e.g. sodium lauryl sulfate, sodium N-lauroyl sarcosinate, etc.), extracting the cells thus treated with an organic solvent (e.g. phenol, chloroform, diethyl ether, etc.) to remove proteins, and then precipitating the DNA with ethanol [cf. Journal of Molecular Biology, Vol. 3, 208 (1961), and Biochimica et Biophysica Acta, Vol. 72, 619 (1963)].

The recombinant plasmid comprising the chromosomal DNA containing an esterase secretory gene and the vector plasmid DNA can easily be prepared by a conventional method, for example, by cleaving the chromosomal DNA and the plasmid

DNA with an appropriate restriction endonuclease (e.g. *Eco*RI, *Bam*HI, *Hin*dIII, *Sal*I, *Sac*I, etc.) and then treating the resultant with DNA ligase (e.g. T4 DNA ligase, *E. coli* DNA ligase, etc.), if required, after treating the resultant with a terminal transferase or DNA polymerase, subjecting to said treatment with DNA ligase [cf. Methods in Enzymology, Vol. 68, 41 (1979), and Yasuyuki Takagi, "Procedure for Experiment in Genetic Engineering", page 135, published by Kodansha, 1980].

Selection of the desired recombinant plasmid containing the esterase secretory gene from a mixture of recombinant plasmids obtained by the above procedure can be done as follows.

Microbial cells being restriction endonuclease deficient and having esterase productivity, for example *Escherichia coli* K12 DH5 harboring a recombinant plasmid which contains an esterase gene (e.g. pLIPE111 disclosed in Japanese Patent First Publication (Kokai) No. 344891/1993, etc.) are transformed with the esterase-secretory gene-containing recombinant plasmids, and the thus-transformed cells are spread onto an agar medium containing an emulsified triglyceride in which the cell can grow, for example, a nutrient agar medium containing tributyrin emulsified with polyoxyethylene cetyl alcohol ether (Brij 58) and also containing a prescribed concentration of an antibiotic. After incubation at 30 to 37°C for 1 to 2 days, the colony of a transformant around which a large clear zone is formed is isolated.

The above introduction of the recombinant plasmid

into the host microorganism is carried out by a conventional method, for example, by treating the host cells with an aqueous calcium chloride solution at a low temperature to increase the membrane permeability of the cells and then introducing the recombinant plasmid into the host cells [cf. Journal of Molecular Biology, Vol. 53, 159 (1970)], or by an electroporation method.

The desired transformant may also be selected by the procedure comprising transforming cells of a microorganism (e.g. *Escherichia coli* K12 DH5, etc.) with a recombinant plasmid produced by inserting both of a DNA participating in the esterase secretory mechanism and of an esterase gene into a single vector plasmid, and then treating the resultant transformed cells in the same manner as described above.

Then, the plasmid DNA is extracted from the transformant by "alkaline lysis method" to give the recombinant plasmid, i.e. a plasmid produced by inserting an esterase gene of *Serratia marcescens* into a vector plasmid.

In order to make the transformation efficient, the recombinant plasmid thus obtained is modified in a microorganism which is restriction endonuclease deficient and is the same species of the host microorganism to be used for expressing the esterase. That is, when the host microorganism to be used is *Serratia marcescens* Sr41, the recombinant plasmid obtained above is introduced into *Serratia marcescens* TT392, which is restriction enzyme deficient strain. The recombinant plasmid thus modified is isolated from the

microorganism. The modified recombinant plasmid is then introduced into a host microorganism to obtain the desired transformant suitable for the production of the desired esterase.

The introduction of the recombinant plasmid into the host microorganism can easily be done by the method of Takagi & Kizumi [cf. Journal of Bacteriology, Vol. 161, 1 (1985)], and the isolation of the plasmid can be done, for example, by alkaline lysis method. Besides, the desired transformant may be obtained by isolating the colony expressing antibiotics resistance. The host microorganisms to be used for said transformation include the above-mentioned microorganisms but are preferably strains having high esterase productivity.

The transformed microorganisms obtained by the above-mentioned method are, for example, *Serratia marcescens* TA5030 which is obtained by introducing a recombinant plasmid into *Serratia marcescens* Sr41 wherein said recombinant plasmid being obtained by inserting an about 2.6 kb *Sal*I-*Bst*PI DNA fragment containing an esterase gene and an about 6.5 kb *Eco*RV-*Eco*RV DNA fragment containing an esterase secretory gene into a vector plasmid pMW119; *Serratia marcescens* TBS90 which is obtained by introducing a recombinant plasmid into *Serratia marcescens* Sr41 wherein said recombinant plasmid being obtained by inserting an about 2.6 kb *Sal*I-*Bst*PI DNA fragment containing an esterase gene and an about 9.0 kb *Bam*HI-*Sac*I DNA fragment containing an esterase secretion gene into a vector plasmid pMW119. These transformed strains all have the same

morphological characteristics as those of the host microorganism *Serratia marcescens* Sr41.

The production of esterase with the transformed microorganism obtained above is carried out by cultivating the microorganism in a medium and collecting the esterase outside and inside the cells of the microorganism.

The medium used for the production of esterase includes any conventional medium wherein the microorganism can grow. Suitable medium contains a carbon source such as saccharides (e.g. glucose, sucrose, molasses, etc.), organic acids (e.g. fumaric acid, citric acid, etc.), alcohols (e.g. glycerol, etc.), or amino acids (e.g. alanine, glutamine, asparagine, etc.) and a nitrogen source such as inorganic ammonium salts (e.g. ammonium sulfate, ammonium chloride, etc.), urea, peptone, corn steep liquor, yeast extract, casein hydrolysate, and the like. The carbon source is usually contained in an amount of 1 to 15 (W/V)% based on the medium, and the nitrogen source is usually contained in an amount of 0.1 to 2.0 (W/V)% based on the medium. The medium may optionally contain further an appropriate amount of an inorganic salt (e.g. phosphate, magnesium salt, potassium salt, calcium salt, etc.) and/or a metallic ion (e.g. iron, manganese, copper, zinc, etc.). In case of a synthetic medium, it may further contain vitamins or amino acids, and further, inducers for esterase production (e.g. vegetable oils, surfactants, etc.), defoaming agents, antibiotics which are suitable for stabilizing the recombinant plasmid in micro-

organisms. The medium is preferably adjusted to a pH 5 to 8.

The cultivation of the transformed microorganism is carried out by a conventional method. For example, the microorganism is inoculated into a medium and is cultivated by shaking culture, aeration culture, standing culture, continuous culture, or the like. The cultivation conditions may vary depending on the kinds of the medium and cultivation methods, but may be any conditions suitable for growth of the microorganism, usually at the initial pH 5 - 8, at 20 to 40°C, for 1 to 2 days.

The esterase produced outside and inside the cultivated cells is collected by a conventional method. For example, the esterase contained in the medium is collected by means of salting out with an inorganic salt, precipitation with an organic solvent, absorption or desorption with ion exchange resin and various column chromatography, gel filtration, use of protein-precipitating agent, or a combination of these methods. The esterase accumulated within the cells is obtained by firstly disrupting the cells by a physical method such as frictional disrupting device (Dyno Mill) or a chemical means such as treatment with lysozyme, and then collecting the esterase in the cell extract by the above-mentioned method.

The esterase secretory gene of this invention is not limited to those of the DNA sequences disclosed specifically in the present specification but includes any gene having a DNA sequence obtained by modifications in the sequence such

as insertion, deletion or substitution. That is, the esterase secretory gene may artificially be modified directly in a test tube by using a synthetic mutated DNA primer designed on the basis of the DNA sequence of the gene encoding an esterase specifically disclosed herein, or by using a chemical mutating agent such as formic acid, hydrazine sulfite. Further, a mutant gene may be obtained by treating an esterase producing strain with NTG or UV.

Examples

The present invention is illustrated by the following Examples but should not be construed to be limited thereto.

In the Examples, the esterase activity was measured by a convenient method (using Lipase Kit S wherein the substrate being dimercaprol tributyrate, manufactured by Dainippon Pharmaceutical Co., Ltd., Japan). It may also be measured by another method comprising subjecting the product to enzymatic reaction in olive oil (as a substrate) at pH 8.0, 37°C for 20 minutes, and then measuring the amount of formed fatty acid, wherein the unit of esterase activity is expressed as μmols of fatty acid formed per minute.

Besides, the medium used in Examples has the following formulation, wherein "%" is W/V% unless specified otherwise.

LB medium: 1.0% of Bactotryptone (manufactured by Difco), 0.5% of Bacto Yeast Extract (manufactured by Difco), and 0.5% of sodium chloride.

LBG plate medium: 1.0% of Bactotryptone (manufactured by Difco), 0.5% of Bacto Yeast Extract (manufactured by Difco), 0.5% sodium chloride, and 1.0% of Gellan Gum (manufactured by Wako Pure Chemical Industries, Ltd., Japan).

Tributyrin-containing LBG plate medium: LBG plate medium containing 0.5 v/v% of tributyrin, 0.5% of polyoxyethylene cetyl alcohol ether, and 0.005% of ampicillin.

Esterase producing medium: 1.0% of dextrin, 2.0% of meast, 0.2% of ammonium sulfate, 0.1% of potassium dihydrogen phosphate, 0.05% of magnesium sulfate heptahydrate, 0.01% of calcium chloride dihydrate, 0.001% of ferrous sulfate heptahydrate, 0.5% of Tween 80, and 0.1% of colorin.

Example 1

(1) Preparation of chromosomal DNA containing an esterase secretory gene:

Serratia marcescens Sr41 (FERM BP-487) was subjected to aerobic shaking culture in LB medium (200 ml) at 30°C overnight, and then the cells were collected by centrifugation. The cells were suspended in 0.9% aqueous sodium chloride solution (200 ml) once and then collected by centrifugation in order to wash them. The cells thus washed were suspended in an aqueous solution of 50mM Tris-HCl-50mM disodium ethylenediamine tetraacetate (pH 7.5, 200 ml) containing 200 mg of lysozyme, and the mixture was allowed to stand at room temperature for one hour.

To the mixture was added sodium lauryl sulfate in a concentration of 0.5%, and thereto was further added

Protenase K, and the mixture was mildly shaken at 50°C for 3 hours to lyse the cells. The mixture was extracted twice with an equal volume of phenol saturated with an aqueous solution of 10 mM Tris-HCl-1 mM disodium ethylenediamine tetraacetate (pH 8.0) (hereinafter, referred to as "TE"), and further extracted twice with a mixture of equal volume of TE-saturated phenol and chloroform, and then the resultant aqueous phase was subjected to precipitation with ethanol. The precipitate was dissolved in TE to prepare a TE solution containing 2.5 mg of the chromosomal DNA containing an esterase secretory gene.

(2) Preparation of a recombinant plasmid DNA:

The chromosomal DNA prepared in the above (1) (20 µg) was completely digested with restriction endonuclease *Sac*I, which was extracted twice with a mixture of equal volume of TE-saturated phenol and chloroform. To the mixture was added 0.5 volume of 7.5 M ammonium acetate, and the DNA was recovered by precipitating with two times volume of ethanol.

A plasmid vector pMWE121 was prepared by inserting an about 2.6 kb genome DNA fragment (*Sal*I-*Bst*PI fragment) containing an esterase gene originated from *Serratia marcescens* Sr41 into a vector plasmid pMW119. DNA (0.5 µg) of said plasmid vector pMWE121, which was completely digested with the same restriction enzyme as above, was dephosphated by treating with alkaline phosphatase (manufactured by Takara Shuzo Co., Ltd., Japan, 0.4 unit) at 56°C for one hour, and the resultant was extracted twice with a mixture of equal

volume of TE-saturated phenol and chloroform, and thereto was added 0.5 volume of 7.5M ammonium acetate, and then the vector plasmid DNA was recovered by precipitating with two times volumes of ethanol. The dephosphated plasmid vector thus obtained was mixed with the chromosomal DNA obtained above (3 µg) and ligated with a DNA ligation kit (manufactured by Takara Shuzo Co., Ltd.) at 4°C for 16 hours to give the recombinant plasmid DNA.

(3) Transformation with the recombinant plasmid and preparation of colony bank with *E. coli* host:

The cells of *E. coli* DH5 were treated by the method of Hanahan [cf. Journal of Molecular Biology, Vol. 166, 557 (1983)] and thereto was added the reaction mixture containing a plasmid DNA obtained in the above (2), by which the transformation was effected. The cells thus treated were spread onto LBG plate medium containing ampicillin (50 µg/ml), and they were incubated at 37°C overnight to give the transformant (about 50,000 strains) containing recombinant plasmids inserted with fragments of the chromosomal DNA of *Serratia marcescens* Sr41.

(4) Isolation and identification of a transformant strain containing an esterase secretory gene:

When an esterase producing strain is inoculated to a tributyrin-containing LBG plate medium, the esterase produced in the medium decomposes tributyrin to give fatty acids, by which the triglyceride emulsion around the colony is modified to form a circular clear zone around the colonies.

By utilizing this phenomenon, the screening of transformants was effected.

The transformants (about 50,000 strains) obtained in the above (3) were inoculated to a tributyrin-containing LBG plate medium, which was incubated at 37°C overnight, by which one strain forming a clear zone was isolated from the DNA bank of *Sac*I.

The formation of this clear zone will be owing to the increase of ability of extracellulor secretion of esterase by introducing the recombinant plasmid containing an esterase secretory gene, because when *Serratia marcescens* Sr41 was inoculated to a tributyrin-containing LBG plate medium and incubated at 37°C overnight, the formation of a clear zone was observed, but when the untransformed *E. coli* DH5 and the *E. coli* DH5 carrying a plasmid vector pMWE121 were inoculated to a tributyrin-containing LBG plate medium and incubated at 37°C overnight, no formation of a clear zone was observed.

Besides, the transformant being capable of formation of a clear zone was cultivated in a LB medium (60 ml) containing ampicillin (200 μg/ml) by aerobic shaking culture at 37°C overnight, and the esterase activity of the supernatant of the culture mixture was measured by the convenient method. As a result, there was observed 34,800 units of esterase activity.

Moreover, the supernatant of the above culture mixture was subjected to an electrophoresis with SDS polyacrylamide gel, and the resultant was subjected to Western

blotting analysis by using rabbit antiesterase antibody to an esterase produced by *Serratia marcescens*. As a result, the product showed a new band, which was not shown in the product from *E. coli* DH5 containing only vector plasmid, at the same position as that in the purified standard esterase obtained from the supernatant of the culture of *Serratia marcescens* Sr41.

Example 2

Analysis of plasmid:

A plasmid DNA was prepared from the cells of the transformant obtained in Example 1-(4) by a conventional method [cf. Maniatis et al., "Molecular Cloning", page 368, Cold Spring Harbor Laboratory, U.S.A. (1982)], cleaved with various restriction endonucleases and then subjected to an agarose gel electrophoresis. As a result, it was confirmed that this plasmid (hereinafter, referred to as "pKHE200") contained a *Sac*I DNA fragment of about 20.0 kb in addition to about 2.6 kb *Sal*I-*Bst*PI DNA fragment containing an esterase gene originated from *Serratia marcescens*.

The restriction endonuclease map of the *Sac*I DNA fragment of about 20.0 kb contained in said pKHE200 is shown in the accompanying Fig. 1.

The *Sac*I DNA fragment of about 20.0 kb of the plasmid pKHE200 was cleaved with various restriction endonucleases, and each DNA fragment was subcloned into plasmid vector pMWE121, followed by transformation of *E. coli* DH5 with the recombinant plasmid thus obtained. The transformants were

cultivated in a tributyrin-containing LBG plate medium in the same manner as described above, and it was determined whether a clear zone was formed or not.

The restriction endonuclease maps of each DNA fragment are shown in the accompanying Fig. 1. As to *E. coli* DH5 transformed with the plasmid containing *Bam*HI-*Sac*I DNA fragment of about 9.0 kb (pKHE90) and the plasmid containing *Eco*RV-*Eco*RV DNA fragment of about 6.5 kb (pKHE65), there were observed the formation of a clear zone and esterase activity (measured by the convenient method). However, as to *E. coli* DH5 transformed with the plasmid containing *Sac*I-*Bam*HI DNA fragment of about 7.5 kb (pKHE75) and with the plasmid containing *Bam*HI-*Bam*HI DNA fragment of about 3.5 kb (pKHE35), there was observed neither the formation of a clear zone nor esterase activity (measured by the convenient method).

It was found from the above results that the esterase secretory gene originated from *Serratia marcescens* Sr41 was present in the *Eco*RV-*Eco*RV DNA fragment of about 6.5 kb.

The *E. coli* DH5 transformed with the plasmid pKHE65 has been deposited to National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology on February 9, 1996 as accession number FERM BP-5385 (designated "*Escherichia coli* TA5029").

Example 3

Analysis of clone DNA:

[Determination of nucleotide sequence]

The recombinant plasmid pKHE65 was treated with Kilobase Deletion Kit (manufactured by Takara Shuzo Co., Ltd.) to prepare various deletion plasmids. These plasmids thus obtained were subjected to annealing of a primer, synthesis of complementary chain with Klenow fragment of DNA polymerase [labelled with (α-^{32}P)dCTP (14.8 x 10^6 Bq/pmol, 74 x 10^4 Bq)] according to the dideoxy chain termination method of Sanger et al. [Proc. Natl. Acad. Sci. USA, Vol. 74, 5463 (1977)], and the nucleotide sequences thereof were determined based on the data of an electrophoresis with an 8% urea-modified polyacrylamide gel and autoradiography.

As a result, it has been found that the DNA sequence of the esterase secretory gene originated from *Serratia marcescens* Sr41 is composed of 4466 base pairs from the initiation codon GTG to the codon GAG as shown in SEQ ID NO:1, which contains DNA regions (ORF) coding for three kinds of polypeptides which participate in the mechanism of secretion of esterase. (It is assumed that these three ORFs compose a pair of operon.)

Besides, the amino acid sequences of the polypeptides coded by each ORF are shown in SEQ ID NO: 2, 3 and 4, respectively.

Example 4

Preparation of a strain having high esterase productivity:

The plasmid pKHE65 was introduced into a restriction

endonuclease-deficient strain, *Serratia marcescens* TT392, to give a transformed strain. The pKHE65 plasmid DNA modified with *Serratia marcescens* was extracted from the cells of the transformed strain by "alkaline lysis method". Then, *Serratia marcescens* Sr41 cells were transformed with the plasmid DNA obtained above by electroporation method to give a transformant *Serratia marcescens* TA5030). The thus-obtained transformant (one platinum loop) was inoculated to an esterase-producing medium containing ampicillin (500 μg/ml) and subjected to reciprocating shaking culture (shaking amplitude 7 cm, 120 r.p.m.) at 30°C for 20 hours. The culture broth was centrifuged to give a supernatant having an esterase activity of about 3.5×10^5 unit/ml (measured by the convenient method). This strain had about 10 times higher esterase productivity than the host strain *Serratia marcescens* Sr41 and further about 2 times higher esterase productivity than *Serratia marcescens* Sr41 containing recombinant plasmid pMWE121 (used as a vector plasmid).

Effects of the Invention

The microorganism transformed with a recombinant plasmid containing an esterase secretory gene of the present invention have remarkably excellent capability of extracellular secretion of esterase. Accordingly, when an esterase-producing microorganism harboring a recombinant plasmid which contains an esterase gene is further transformed with the recombinant plasmid which contains the esterase

secretory gene of the present invention, there can be obtained a microorganism having excellent properties in both of esterase productivity and extracellulor secretion of esterase, and the cultivation of said transformant can give the desired esterase on an industrial scale.

Sequence Listing

SEQ ID NO:1

LENGTH: 4466 base pairs

TYPE: nucleic acid

STRANDEDNESS: double

TOPOLOGY: linear

MOLECULE TYPE: genomic DNA

STRAIN: Serratia marcescens Sr41

SEQUENCE

GTG AAT CAA TTT ATC CCG CGC AAC GAA ATT GCG GAT GTT ATA CGT ACA 48

CGC AGC AAA GTC TTC TGG ACC GTT GGT ATA TTT ACT GCG TTT ATT AAC 96

CTG TTA ATG CTG GTT CCT TCC ATT TAT ATG CTC CAG GTT TAC GAC CGG 144

GTG CTG CCT TCG CGC AAT GAA ATC ACG CTG TTA ATG CTG ACG CTG ATC 192

ATG CTG GGC ATG TTC GGC ATG ATG TCG CTG TTG GAA TAC GTG CGC AGC 240

ATG GTG GTG ATC CGC ATC GGC AGC CAG CTG GAT ATG CGT CTC AAC ACG 288

CGA GTC TAT ACC GCG GCC TAC GAA GCG AAT CTG AAA AAC GGT TCG TCT 336

GAC GCC GGT CAG ATG CTG AGC GAT TTG ACC AAT CTG CGC CAA TTC CTC 384

ACC GGT AGC GCG CTG TTC GCC TTC TTT GAT GCG CCG TGG TTT CCG ATC 432

TAT CTG TTG GTG ATA TTC CTC TTT AAC CCT TGG TTG GGC CTT TTC GCC 480

CTG GTC GGT GCG CTG TTG CTG ATC GCA TTG GCG GTA ATC AAT GAA GTG 528

GTT TCG AAA AAG CCG CTG GGA GAA GCC AGC AAG CTG TCG ATC ATG TCA 576

GGT AAT TTG GCC AGC ACC AAT CTG CGA AAT GCC GAA GTG ATC GAG GCT 624

TTG GGG ATG TTG CCT AAC CTG AAA CGC CGG TGG TTC GGT CTG CAC CAG 672

CGG TTC TTG AAC AGC CAA CGC ATC GCC AGC GAA CGC GCA TCG CGG GTC 720

ACG TCA ATC ACC AAG TTC GTG CGT ATG TCG CTG CAG TCC TTA GTG TTG 768

GGC CTG GGG GGA TGG TTG GCG ATT GAT GGG CAC ATC ACG CCC GGC ATG 816

ATG ATC GCC GGT TCT ATA TTG ATG GGG CGA ACG TTG GCG CCG ATC GAG 864

CAG GTC ATT AAC GTT TGG AAA AGC TAT AGC GCG GCC AAA CTT TCT TAT 912

GGC CGC TTG GTC AAG CTG CTG GAA ACG CAT CCG CAG CGT GGT ACC GGC 960

ATG TCG CTG CCG CGT CCG GAA GGT GTG CTC TCC GTA GAA GGC GTG ACC 1008

GCC ACG CCT CCG GGA TCG AAA GGG GAT GCG GTG CTG CAT AAC GTA AGT 1056

TTT GCC ATT CAA CCC GGC GAT GTG CTG GGG ATT ATC GGT CCG AGT GCG 1104

TCG GGC AAA TCA ACA TTG GCG CGC TTA CTG GTC GGT ATT TGG CCT GTG 1152

AGC GAA GGG ATA GTG CGG TTG GAT AAT GCC GAC ATC TAC CAG TGG AAC 1200

AAA GAC GAA CTG GGG CCC TAT ATC GGC TAT CTG CCG CAG GAC ATC GAG 1248

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TTG TTC GCC GGC ACT ATC GCC GAG AAC ATC GCT CGC TTT AAC GAC ATC  1296
GAT TCA GAG AAA GTG ATT GAG GCT GCC AAG CTG GCT GGT GTG CAT GAA  1344
CTG ATC CTG CGT TTC CCT AAC GGT TAC GAT TCG GTG ATC GGC AAC GGT  1392
GGT GCA GGG TTG TCC GGC GGG CAG AAG CAA CGT ATC GGC CTG GCG CGG  1440
GCA TTG TAT GGC GAT CCC GCG TTG GTG GTG TTG GAT GAG CCT AAC TCC  1488
AAC CTG GAT GAT GCC GGC GAG AAA GCG TTG AAC CAG GCC ATC ATG TTC  1536
CTT AAA CAG CGT AAT AAG ACG GTG GTC CTG ATC ACT CAC CGC ACC AAT  1584
CTG CTG TCG ATG ACC AGC AAG CTG TTG CTG TTG GTT AAC GGG AAC GTC  1632
AAT GCA TTC GGC CCA ACG CAG CAG GTG CTG CAG GCG TTG GCG AAT GCG  1680
CAA AAA GCG CAG GTG CCT CCG CAG GCG GTG CGT GCG GTG AAC TCC GAG  1728
CCG GAT GAA GGC GAA ATC CCT AAA ACT CAA ATT AAT TAA GCCGTGAACT   1777
TGCCCGGCGG CGCTTTTGCG TCGCCGACAG TCAAAGGAGT TGGT ATG TCT ACG CAT 1833
ATT GGC GAG CCG CAA GAC TCG TAT ACT GAA GAG ATC CCA CAA GAT GAA  1881
CGG CGG TTT ACC CGT ATG GGG TGG CTG GTG GTC GGG ATC GGT CTG TTC  1929
GGG TTT TTA GCC TGG GCG GCC TTT GCG CCG TTG GAT AAA GGG GTG GCG  1977
TCG CCG GGA TCG GTA ACC GTT TCC GGC AAC CGC AAA ACG GTG CAG GCC  2025
CCG GCC AGC GGC ATC ATT AAG AAT ATT GCG GTC AGA GAT GGC GAC AAA  2073
GTG AAA GCC GGT GAG GTG CTG GTG CAG CTC AGC CAG GTG CAG GCT CAA  2121
GCT CAG GTT GAT TCG CTG CGG GAT CAG TAC TAC ACC ACG CTG GCG ACA  2169
GAA GGG CGC TTG CTG GCA GAA CGC GAT GGG TTG AGC ATA GTG ACT TTC  2217
TCA CCC ATT TTG GAC GCG GTG AAA GAT AAA CCT CGC GTG GCA GAA ATC  2265
ATT GCA TTG CAA ACG CAG CTG TTC GCC TCC CGC CGC CAA GCG CTG CAA  2313
AGT GAA ATC GAC GGC TAT AAG CAG TCA ATG GAC GGA ATC CGT TTC CAA  2361
TTA AAA GGA CTG CAG GAT TCG CGC GGT AAC AAA CAG ATC CAG CTT TCC  2409
AGC CTG CGT GAG CAG ATG AAC AGC ATG AAG CAG TTG GCG GCG GAC GGT  2457
TAC CTA CCG CGT AAC CGT TAC CTG GAA GTG CAG CGC CAG TTT GCC GAG  2505
GTA AAT AGC AGC ATT GAT GAA ACG GTG GGG CGG ATT GGC CAA TTG CAA  2553
AAG CAG TTG CTG GAA TCA CAG CAA CGC ATC GAT CAG CGT TTC GCC GAC  2601
TAC CAG CGC GAA GTC AGA ACG CAG CTG GCG CAA ACT CAA ATG GAC GCC  2649
AGC GAA TTC CGC AAC AAG CTG CAA ATG GCC GAT TTC GAT CTG GGC AAC  2697
ACC GCC ATC ACC TCA CCG GTG GAC GGC ACC GTG GTT GGA TTG AAT ATC  2745
TTC ACT CAG GGG GGC GTC GTG GGA GCG GGT GAC CAC CTG ATG GAC GTT  2793
GTG CCC AGC CAG GCG ACT TTG GTG GTG GAT TCT CGC CTC AAA GTC GAC  2841
CTG TTC GAT AAG GTG TAC AAC GGG TTG CCG GTG GAT CTG ATG TTT ACC  2889
GCC TTC AAC CAA AAC AAA ACC CCG AAA ATT CCG GGA ACC GTC ACC TTG  2937
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GTT TCC GCC GAC CGC CTG GTC GAC AAA GCC AAT GGC GAA CCT TAC TAC  2985
CAG ATG CAG GTC ACG GTC TCG CCG GAG GGC ATG AAA ATG CTC AGT GGC  3033
GAG GAC ATC AAG CCG GGG ATG CCG GTG GAG GTG TTC GTG AAA ACG GGG  3081
TCG CGC TCG CTG TTG AGC TAT CTG TTT AAA CCT ATT TTG GAT CGC GCT  3129
CAT ACT TCA TTA ACC GAG GAA TAA TT TTG ATT CAT TCA AAA CGA CAG   3176
GCT GCC GGT CTG GTT ATC GGC ACC CTT TTG TTT GCG ATG TCT GCG CCG  3224
GTT TAT TCG ATA GGG ATT TTA GAC GCA TAT TCG CTG GCA TTA GAA AAG  3272
GAC CCG ACC TTT CGG GCG GCT ATA AAA GAG AAA GAA GCG GGA GAT GAA  3320
AAC GAA AAT ATC GGC AGG GCA GGG CTG CTG CCG AAG GTA TCG CTG AAC  3368
TAC CAG AAT TCG CCG CGC AAC TGG CAA ACT CAG AAG TAC CCG CAA AGC  3416
GAC TTT TTC GGC AAT GTT TCG GAG GTT ACC CGG CGG CAG CAA TAT CGC  3464
AGC TAT TCC AGT TCG ATC ACC TTG ACG CAG CCG CTG TTC GAT TAT GAA  3512
GCT TAC GCC AGG TAC AAA GCC GGC GTG GCG CAG ACC ATG ATG TCG GAC  3560
GAG ACG TAT CGC GGT AAG TTG CTG GAT TTG GCG GTG AGG GTG ATT AAC  3608
GCC TAT GTC GAA GTG GCT TAT TCC AAG GAT CAA ATC GCG TTG GCC GAA  3656
GCT CAA AAG GCG GCT TAC AAG GAA CAG CTG ACT TTG AAC GAT CGC CTG  3704
ATG AGC GCC GGT GAA GGT ACC ATT ACC GAC GTA TCC GAA ACT CAG GCG  3752
CGC TAT AGC CTG GCT GAA GCA CAG GTG ATA GAA GCG CGC GAT GCA CTG  3800
GAT GCC GCA CAG CGT GAA TTG GAA GTA ATT ATC GGC ATG CCG CTG AAC  3848
CAA CTG GAT GAA TTG CAG GTC TTG CGG CCG GGT AAA TTC AAA GTG GCG  3896
CCG TTA ATC CCG TCC AAG TTC GAA GAG TGG CAA AAG ATC GCG TTG GAG  3944
AAC AAC CCT GTA TTG GCC GCC TCG CGT CAT GGC GTG GAT GCT GCT AAG  3992
TAT GAT GTC GAA AGG AAA CGG GCT GGC TTT ATG CCA CAG GTT CAG CTG  4040
TAT GCT TCG CAT TCG GAA AAC GAC GCC AGC AGC GAC AAC ACG GTT AAC  4088
CAG AAA TAC CGT ACT GAC AGC ATT GGC GTG CAG GTC AGC ATG CCT ATT  4136
TAT TCC GGT GGC GGT GTT TCG GCA TCG ACC CGC CAG GCA GCG GCG CGT  4184
TAC GGG CAA GCG ATG TAT GAA ATG GAT GCG CAA ACG GGC ACC ACG CTC  4232
AAC GAT CTG CGC AAA CAG TAC AAT TTG TGT ATT AGC AGC AGC GCT AAA  4280
GTG GCG GCC TAT GAA CTG GCG GTT CAA TCG GCG ACG ACC CAG GTG ACG  4328
GCG ACC CGG CAA AGC GTG CTG GCT GGG CAA CGT GTC AAC GTC GAT GTG  4376
CTC AAT GCC GAA CAG CAG CTC TAT AGC GCA CAG GCG ATT TTG GCC TCT  4424
GCT AAA TAC ACT TAT ATC AAA TCC TGG GAT CAC CCT ATT GAG          4465
```

SEQ ID NO:2

LENGTH: 588 amino acids

TYPE: amino acid
TOPOLOGY: linear
MOLECULE TYPE: peptide
SEQUENCE

```
Val Asn Gln Phe Ile Pro Arg Asn Glu Ile Ala Asp Val Ile Arg Thr
 1               5                  10                  15
Arg Ser Lys Val Phe Trp Thr Val Gly Ile Phe Thr Ala Phe Ile Asn
            20                  25                  30
Leu Leu Met Leu Val Pro Ser Ile Tyr Met Leu Gln Val Tyr Asp Arg
        35                  40                  45
Val Leu Pro Ser Arg Asn Glu Ile Thr Leu Leu Met Leu Thr Leu Ile
    50                  55                  60
Met Leu Gly Met Phe Gly Met Met Ser Leu Leu Glu Tyr Val Arg Ser
65                  70                  75                  80
Met Val Val Ile Arg Ile Gly Ser Gln Leu Asp Met Arg Leu Asn Thr
                85                  90                  95
Arg Val Tyr Thr Ala Ala Tyr Glu Ala Asn Leu Lys Asn Gly Ser Ser
            100                 105                 110
Asp Ala Gly Gln Met Leu Ser Asp Leu Thr Asn Leu Arg Gln Phe Leu
        115                 120                 125
Thr Gly Ser Ala Leu Phe Ala Phe Phe Asp Ala Pro Tyr Phe Pro Ile
    130                 135                 140
Tyr Leu Leu Val Ile Phe Leu Phe Asn Pro Trp Leu Gly Leu Phe Ala
145                 150                 155                 160
Leu Val Gly Ala Leu Leu Leu Ile Ala Leu Ala Val Ile Asn Glu Val
                165                 170                 175
Val Ser Lys Lys Pro Leu Gly Glu Ala Ser Lys Leu Ser Ile Met Ser
            180                 185                 190
Gly Asn Leu Ala Ser Thr Asn Leu Arg Asn Ala Glu Val Ile Glu Ala
        195                 200                 205
Leu Gly Met Leu Pro Asn Leu Lys Arg Arg Trp Phe Gly Leu His Gln
    210                 215                 220
Arg Phe Leu Asn Ser Gln Arg Ile Ala Ser Glu Arg Ala Ser Arg Val
225                 230                 235                 240
Thr Ser Ile Thr Lys Phe Val Arg Met Ser Leu Gln Ser Leu Val Leu
```

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                245                 250                 255
Gly Leu Gly Gly Trp Leu Ala Ile Asp Gly His Ile Thr Pro Gly Met
            260                 265                 270
Met Ile Ala Gly Ser Ile Leu Met Gly Arg Thr Leu Ala Pro Ile Glu
        275                 280                 285
Gln Val Ile Asn Val Trp Lys Ser Tyr Ser Ala Ala Lys Leu Ser Tyr
    290                 295                 300
Gly Arg Leu Val Lys Leu Leu Glu Thr His Pro Gln Arg Gly Thr Gly
305                 310                 315                 320
Met Ser Leu Pro Arg Pro Glu Gly Val Leu Ser Val Glu Gly Val Thr
                325                 330                 335
Ala Thr Pro Pro Gly Ser Lys Gly Asp Ala Val Leu His Asn Val Ser
            340                 345                 350
Phe Ala Ile Gln Pro Gly Asp Val Leu Gly Ile Ile Gly Pro Ser Ala
        355                 360                 365
Ser Gly Lys Ser Thr Leu Ala Arg Leu Leu Val Gly Ile Trp Pro Val
    370                 375                 380
Ser Glu Gly Ile Val Arg Leu Asp Asn Ala Asp Ile Tyr Gln Trp Asn
385                 390                 395                 400
Lys Asp Glu Leu Gly Pro Tyr Ile Gly Tyr Leu Pro Gln Asp Ile Glu
                405                 410                 415
Leu Phe Ala Gly Thr Ile Ala Glu Asn Ile Ala Arg Phe Asn Asp Ile
            420                 425                 430
Asp Ser Glu Lys Val Ile Glu Ala Ala Lys Leu Ala Gly Val His Glu
        435                 440                 445
Leu Ile Leu Arg Phe Pro Asn Gly Tyr Asp Ser Val Ile Gly Asn Gly
    450                 455                 460
Gly Ala Gly Leu Ser Gly Gly Gln Lys Gln Arg Ile Gly Leu Ala Arg
465                 470                 475                 480
Ala Leu Tyr Gly Asp Pro Ala Leu Val Val Leu Asp Glu Pro Asn Ser
                485                 490                 495
Asn Leu Asp Asp Ala Gly Glu Lys Ala Leu Asn Gln Ala Ile Met Phe
            500                 505                 510
Leu Lys Gln Arg Asn Lys Thr Val Val Leu Ile Thr His Arg Thr Asn
        515                 520                 525
```

```
Leu Leu Ser Met Thr Ser Lys Leu Leu Leu Leu Val Asn Gly Asn Val
     530                 535                 540
Asn Ala Phe Gly Pro Thr Gln Gln Val Leu Gln Ala Leu Ala Asn Ala
545                 550                 555                 560
Gln Lys Ala Gln Val Pro Pro Gln Ala Val Arg Ala Val Asn Ser Glu
                565                 570                 575
Pro Asp Glu Gly Glu Ile Pro Lys Thr Gln Ile Asn
            580                 585
```

SEQ ID NO:3
LENGHT: 443 amino acids
TYPE: amino acid
TOPOLOTY: linear
MOLECULE TYPE: peptide
SEQUENCE

```
Met Ser Thr His Ile Gly Glu Pro Gln Asp Ser Tyr Thr Glu Glu Ile
 1               5                  10                  15
Pro Gln Asp Glu Arg Arg Phe Thr Arg Met Gly Trp Leu Val Val Gly
            20                  25                  30
Ile Gly Leu Phe Gly Phe Leu Ala Trp Ala Ala Phe Ala Pro Leu Asp
        35                  40                  45
Lys Gly Val Ala Ser Pro Gly Ser Val Thr Val Ser Gly Asn Arg Lys
     50                  55                  60
Thr Val Gln Ala Pro Ala Ser Gly Ile Ile Lys Asn Ile Ala Val Arg
 65                  70                  75                  80
Asp Gly Asp Lys Val Lys Ala Gly Glu Val Leu Vla Gln Leu Ser Gln
                85                  90                  95
Val Gln Ala Gln Ala Gln Val Asp Ser Leu Arg Asp Gln Tyr Tyr Thr
            100                 105                 110
Thr Leu Ala Thr Glu Gly Arg Leu Leu Ala Glu Arg Asp Gly Leu Ser
        115                 120                 125
Ile Val Thr Pro Ser Phe Ile Leu Asp Ala Val Lys Asp Lys Pro Arg
    130                 135                 140
Val Ala Glu Ile Ile Ala Leu Gln Thr Gln Leu Phe Ala Ser Arg Arg
145                 150                 155                 160
```

Gln Ala Leu Gln Ser Glu Ile Asp Gly Tyr Lys Gln Ser Met Asp Gly
165 170 175
Ile Arg Phe Gln Leu Lys Gly Leu Gln Asp Ser Arg Gly Asn Lys Gln
180 185 190
Ile Gln Leu Ser Ser Leu Arg Glu Gln Met Asn Ser Met Lys Gln Leu
195 200 205
Ala Ala Asp Gly Tyr Leu Pro Arg Asn Arg Tyr Leu Glu Val Gln Arg
210 215 220
Gln Phe Ala Glu Val Asn Ser Ser Ile Asp Glu Thr Val Gly Arg Ile
225 230 235 240
Gly Gln Leu Gln Lys Gln Leu Leu Glu Ser Gln Gln Arg Ile Asp Gln
245 250 255
Arg Phe Ala Asp Tyr Gln Arg Glu Val Arg Thr Gln Leu Ala Gln Thr
260 265 270
Gln Met Asp Ala Ser Glu Phe Arg Asn Lys Leu Gln Met Ala Asp Phe
275 280 285
Asp Leu Gly Asn Thr Ala Ile Thr Ser Pro Val Asp Gly Thr Val Val
290 295 300
Gly Leu Asn Ile Phe Thr Gln Gly Gly Val Val Gly Ala Gly Asp His
305 310 315 320
Leu Met Asp Val Val Pro Ser Gln Ala Thr Leu Val Val Asp Ser Arg
325 330 335
Leu Lys Val Asp Leu Phe Asp Lys Val Tyr Asn Gly Leu Pro Val Asp
340 345 350
Leu Met Phe Thr Ala Phe Asn Gln Asn Lys Thr Pro Lys Ile Pro Gly
355 360 365
Thr Val Thr Leu Val Ser Ala Asp Arg Leu Val Asp Lys Ala Asn Gly
370 375 380
Glu Pro Tyr Tyr Gln Met Gln Val Thr Val Ser Pro Glu Gly Met Lys
385 390 395 400
Met Leu Ser Gly Glu Asp Ile Lys Pro Gly Met Pro Val Glu Val Phe
405 410 415
Val Lys Thr Gly Ser Arg Ser Leu Leu Ser Tyr Leu Phe Lys Pro Ile
420 425 430

```
Leu Asp Arg Ala His Thr Ser Leu Thr Glu Glu
        435                 440
```

SEQ ID NO:4
LENGTH: 437 amino acids
TYPE: amino acid
TOPOLOTY: linear
MOLECULE TYPE: peptide
SEQUENCE

```
Leu Ile His Ser Lys Arg Gln Ala Ala Gly Leu Val Ile Gly Thr Leu
 1               5                  10                  15
Leu Phe Ala Met Ser Ala Pro Val Tyr Ser Ile Gly Ile Leu Asp Ala
            20                  25                  30
Tyr Ser Leu Ala Leu Glu Lys Asp Pro Thr Phe Arg Ala Ala Ile Lys
        35                  40                  45
Glu Lys Glu Ala Gly Asp Glu Asn Glu Asn Ile Gly Arg Ala Gly Leu
    50                  55                  60
Leu Pro Lys Val Ser Leu Asn Tyr Gln Asn Ser Pro Arg Asn Trp Gln
65                  70                  75                  80
Thr Gln Lys Tyr Pro Gln Ser Asp Phe Phe Gly Asn Val Ser Glu Val
                85                  90                  95
Thr Arg Arg Gln Gln Tyr Arg Ser Tyr Ser Ser Ser Ile Thr Leu Thr
            100                 105                 110
Gln Pro Leu Phe Asp Tyr Glu Ala Tyr Ala Arg Tyr Lys Ala Gly Val
        115                 120                 125
Ala Gln Thr Met Met Ser Asp Glu Thr Tyr Arg Gly Lys Leu Leu Asp
    130                 135                 140
Leu Ala Val Arg Val Ile Asn Ala Tyr Val Glu Val Ala Tyr Ser Lys
145                 150                 155                 160
Asp Gln Ile Ala Leu Ala Glu Ala Gln Lys Ala Ala Tyr Lys Glu Gln
                165                 170                 175
Leu Thr Leu Asn Asp Arg Leu Met Ser Ala Gly Glu Gly Thr Ile Thr
            180                 185                 190
Asp Val Ser Glu Thr Gln Ala Arg Tyr Ser Leu Ala Glu Ala Gln Val
        195                 200                 205
```

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Ile Glu Ala Arg Asp Ala Leu Asp Ala Ala Gln Arg Gln Leu Glu Val
    210                 215                 220
Ile Ile Gly Met Pro Leu Asn Gln Leu Asp Glu Leu Gln Val Leu Arg
225                 230                 235                 240
Pro Gly Lys Phe Lys Val Ala Pro Leu Ile Pro Ser Lys Phe Glu Glu
                245                 250                 255
Trp Gln Lys Ile Ala Leu Glu Asn Asn Pro Val Leu Ala Ala Ser Arg
            260                 265                 270
His Gly Val Asp Ala Ala Lys Tyr Asp Val Glu Arg Lys Arg Ala Gly
        275                 280                 285
Phe Met Pro Gln Val Gln Leu Tyr Ala Ser His Ser Glu Asn Asp Ala
    290                 295                 300
Ser Ser Asp Asn Thr Val Asn Gln Lys Tyr Arg Thr Asp Ser Ile Gly
305                 310                 315                 320
Val Gln Val Ser Met Pro Ile Tyr Ser Gly Gly Gly Val Ser Ala Ser
                325                 330                 335
Thr Arg Gln Ala Ala Ala Arg Tyr Gly Gln Ala Met Tyr Glu Met Asp
            340                 345                 350
Ala Gln Thr Gly Thr Thr Leu Asn Asp Leu Arg Lys Gln Tyr Asn Leu
        355                 360                 365
Cys Ile Ser Ser Ser Ala Lys Val Ala Ala Tyr Glu Leu Ala Val Gln
    370                 375                 380
Ser Ala Thr Thr Gln Val Thr Ala Thr Arg Gln Ser Val leu Ala Gly
385                 390                 395                 400
Gln Arg Val Asn Val Asp Val Leu Asn Ala Glu Gln Gln Leu Tyr Ser
                405                 410                 415
Ala Gln Ala Ile Leu Ala Ser Ala Lys Tyr Thr Tyr Ile Lys Ser Trp
            420                 425                 430
Asp His Pro Ile Glu
        435
```

* C L M S *

GENE PARTICIPATING IN THE MECHANISM OF SECRETION OF ESTERASE

What is claimed is:

1. A gene encoding a polypeptide which participates in the mechanism of secretion of esterase originated from a microorganism of the genus *Serratia*.

2. The gene as claimed in claim 1 which has a DNA sequence as shown in SEQ ID NO: 1.

3. The gene as claimed in claim 1 which comprises a DNA encoding a polypeptide having an amino acid sequence as shown in SEQ ID NO: 2, NO: 3 or NO: 4.

4. A recombinant plasmid comprising a plasmid prepared by inserting the gene as set forth in claim 1 or claim 2 into a vector plasmid.

5. A microorganism transformed with the recombinant plasmid as set forth in claim 4.

6. A transformant microorganism which is obtained by transforming a host microorganism with a recombinant plasmid comprising a plasmid prepared by inserting both of a gene encoding an esterase originated from a microorganism of the genus *Serratia* and a gene encoding a polypeptide which participates in the mechanism of secretion of esterase originated from a microorganism of the genus *Serratia* into a vector plasmid.

7. A transformant microorganism which is obtained by transforming a host microorganism with a recombinant plasmid comprising a plasmid prepared by inserting a gene encoding an esterase originated from a microorganism of the genus *Serratia* into a vector plasmid and a recombinant plasmid

comprising a plasmid prepared by inserting a gene encoding a polypeptide which participates in the mechanism of secretion of esterase originated from a microorganism of the genus *Serratia* into a vector plasmid.

8. The microorganism as claimed in claim 6 or claim 7, wherein the host microorganism is a microorganism of the genus *Serratia* or a microorganism of the genus *Escherichia*.

9. A method for production of an esterase, which comprises cultivating the transformant as set forth in claim 6 or claim 7 in a medium, and collecting the esterase accumulated inside the cells and in the medium.

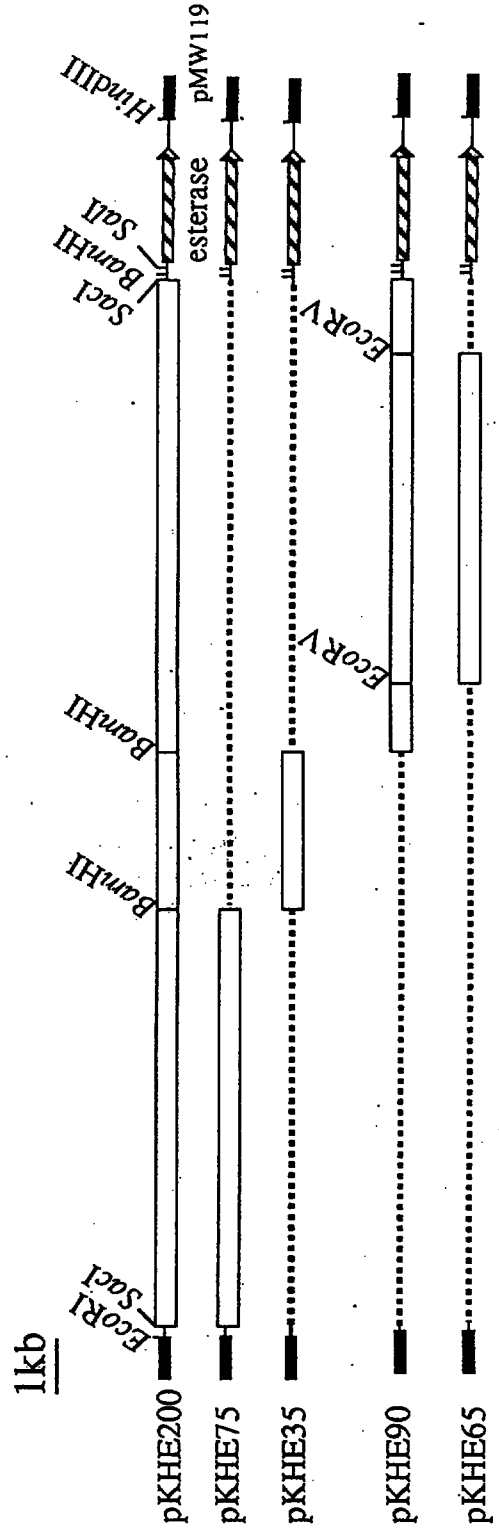
GENE PARTICIPATE IN THE MECHANISM OF SECRETION OF ESTERASE

Abstract of the Disclosure:

A gene encoding a polypeptide which participates in the mechanism of secretion of esterase originated from a microorganism of the genus *Serratia*, a recombinant plasmid comprising a plasmid prepared by inserting said gene into a vector plasmid, a microorganism transformed with said recombinant plasmid, and a method for the production of an esterase which comprises cultivating the transformant microorganism as set forth above in a medium and collecting the produced esterase outside and inside the cells. Said transformed microorganism have remarkably excellent capability of extracellular secretion of esterase.

No drawing is to accompany the abstract upon publication.

FIG 1


1kb
EcoRI
SacI
BamHI
BamHI
SacI
BamHI
SalI
HindIII
pMW119
esterase
EcoRV
EcoRV
pKHE200
pKHE75
pKHE35
pKHE90
pKHE65