

US 20060166312A1

(19) United States (12) Patent Application Publication (10) Pub. No.: US 2006/0166312 A1

Jul. 27, 2006 (43) **Pub. Date:**

Mitani et al.

- (54) NOVEL MICROORGNAISM SENSITIVE TO LYSOZYME
- (75) Inventors: Yasuo Mitani, Hokkaido (JP); Nobutaka Nakashima, Hokkaido (JP); Tomohiro Tamura, Hokkaido (JP)

Correspondence Address: FOLEY AND LARDNER LLP **SUITE 500 3000 K STREET NW** WASHINGTON, DC 20007 (US)

- (73) Assignee: National Institute of Advanced Industrial Science and Technology, Chiyodaku, Tokyo (JP)
- (21) Appl. No.: 10/524,630
- (22) PCT Filed: Aug. 14, 2003
- PCT/JP03/10342 (86) PCT No.:

(30)**Foreign Application Priority Data**

Aug. 20, 2002 (JP)..... 2002-239554

Publication Classification

(51)	Int. Cl.	
. /	C12P 21/06	(2006.01)
	C07K 14/195	(2006.01)
	C12N 1/21	(2006.01)
(50)		125/00 1 4

(52) U.S. Cl. 435/69.1; 435/252.3; 530/350

(57)ABSTRACT

A microorganism of the genus Rhodococcus is provided which has a higher sensitivity to lysozyme at a low concentration than a wild-type strain, which can easily cause cell lysis, and from which a recombinant protein expressed therein is easily recovered. More specifically, a mutant microorganism of the genus Rhodococcus having a higher sensitivity to lysozyme than a wild-type microorganism of the genus Rhodococcus.



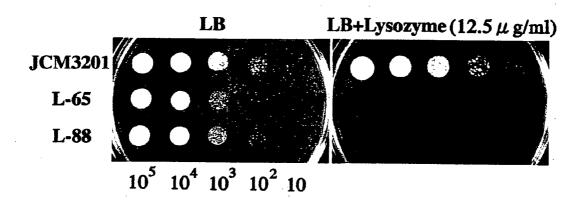


Fig. 2

Rhodococcus erythropolis L-65

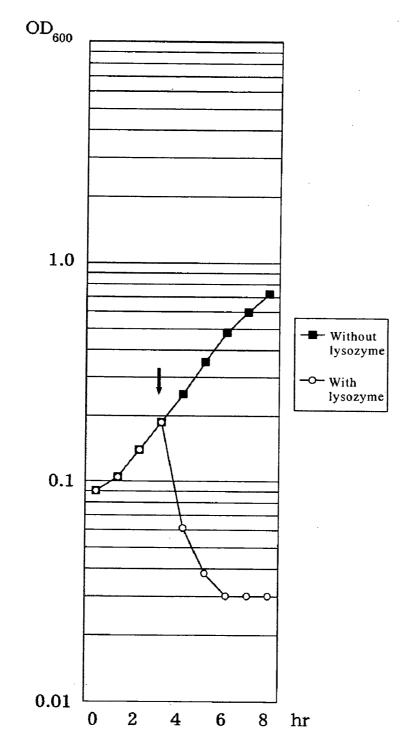


Fig. 3

Rhodococcus erythropolis L-88

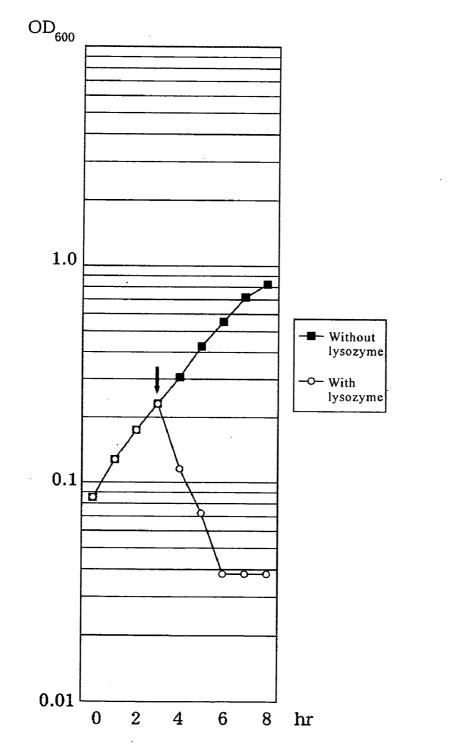
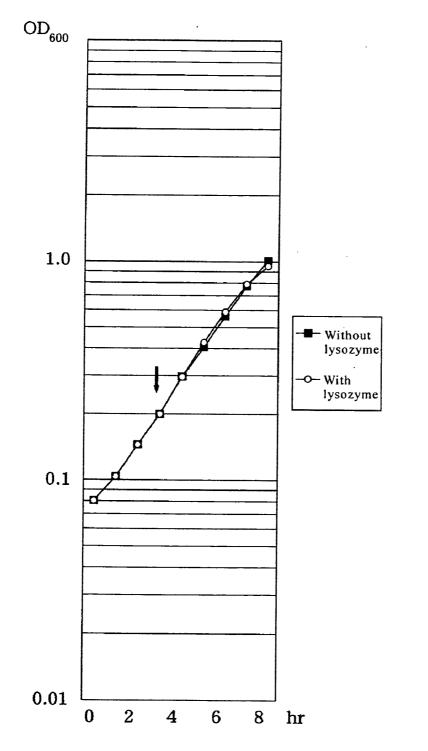
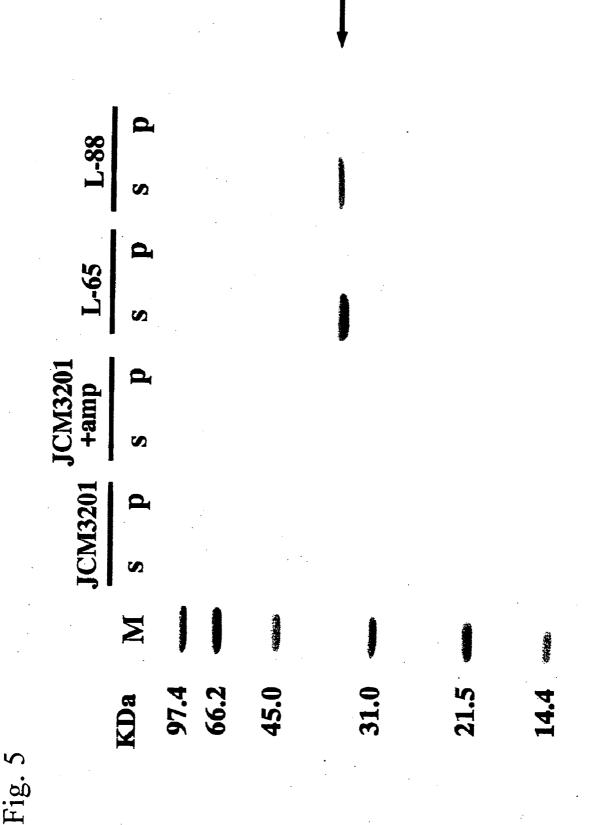


Fig. 4

Rhodococcus erythropolis JCM3201





NOVEL MICROORGNAISM SENSITIVE TO LYSOZYME

TECHNICAL FIELD

[0001] The present invention relates to a microorganism of the genus *Rhodococcus* suitable for production of a recombinant protein, and more specifically, to a mutant strain more sensitive to lysozyme at a low concentration than a wild-type strain and capable of easily causing cell lysis. Use of the mutant strain makes it easier to extract and recover an expressed protein.

BACKGROUND ART

[0002] As a technique for expressing a recombinant protein in a microbial host, an expression system using *Escherichia coli* (*E. coli*) as a host has been generally and widely used. This is because *E. coli* is extremely easy to handle in a laboratory. More specifically, *E. coli* is confirmed as a safe microbial host and proliferates at a high rate, and its molecular biological operations in a laboratory are well established. On the other hand, development of host microorganisms having usefulness and advantages over *E. coli* in view of recombinant protein expression has progressed.

[0003] Microorganisms of the genus *Rhodococcus* are not pathogenic, except a few, and easily cultured in an ordinary laboratory. In addition to such essential conditions, they have the function as microbial catalysts, which is considered to be extremely useful from an industrial point of view. For these reasons, recently, various molecular biological techniques have been developed by use of such microorganisms. For example, in an attempt to add a further useful function to the microorganisms, techniques involving gene recombination has been developed. As a result, a shuttle vector was established which can replicate autonomously both in E. coli and in a microorganism of the genus Rhodococcus (R, De Mot et al., Microbiology 143, 3137-3147, (1997)). Furthermore, there is a report that a transposable transposon is present in a microorganism of the genus Rhodococcus (I, Nagy et al., J. Bacteriol. 179, 4635-4638 (1997)). Thus, it is expected to improve the microorganism in function, for example, by destroying the gene or integrating an exogenous gene into the chromosome.

[0004] In an attempt to further improve a microbial catalytic action based on such a molecular biological establishment, development of a vector for expressing a recombinant protein has been underway (JP Patent Publication (Kokai) No. 10-248578 A (1998)).

[0005] A microorganism of the genus *Rhodococcus*, namely, *Rhodococcus erythropolis* is not only useful as a microbial catalyst but also advantageous in that it can grow under a low temperature condition of 4° C. For this reason, it is expected that *Rhodococcus erythropolis* may produce a recombinant protein or the like in a temperature range where *E. coli* could not be used. Development of an inducible expression vector has been underway for such a purpose (the application already filed by Tamura, on Aug. 12, (2002)).

[0006] However, the cell wall of a microorganism of the genus *Rhodococcus* is particular and rigid in structure compared to those of other gram-positive bacteria. Therefore, extraction of a cellular content from the microorganism is complicated and difficult compared to the case of *E. coli*.

More specifically, a microorganism of the genus Rhodococcus has an extremely strong resistance to a cell-wall lytic enzyme used generally for microbial cell lysis, such as lysozyme. Examples of a cell lysis method include a method of exposing cell wall to a high-concentration antibiotic, such as penicillin, for a predetermined time to weaken the cell wall and then being subjected to cell lysis with lysozyme, and a method of applying ultrasonic treatment to bacterial cells for a long time to physically destroy them. However, these methods are complicated in process, it is difficult to treat a large amount of cells, and specimens are not likely to be treated uniformly. These problems are significant in view of industrial use. The effectiveness of an antibiotic such as penicillin is brought by inhibiting a de-novo synthesis of a cell wall and therefore the cell wall completed in synthesis is not affected by such an antibiotic. Therefore, the effect of such an antibiotic is considered to be low in low-temperature conditions where rapid growth is not expected.

[0007] It is known that the cell wall structure of a microorganism of the genus *Rhodococcus* is commonly seen in bacteria of the genus *Corynebacterium* (C. E. Barry III et al., Prog. Lipid Res. 37, 143-179 (1988)) and an invention similar to the present invention has been made in view of an object of facilitating a molecular biological operation such as transformation (JP Patent Publication (Kokoku) No. 01-003475 B (1989), T. Hirasawa et al., J. Bacteriol. 182, 2696-2701 (2000)).

DISCLOSURE OF THE INVENTION

[0008] The present invention is directed to providing a microorganism of the genus *Rhodococcus* improved in sensitivity to lysozyme and capable of being lysed with lysozyme at a low concentration, the microorganism allowing recovery of the protein by treatment of the microorganism with lysozyme after an exogenous gene is integrated to the microorganism and allowed to express. Furthermore, the present invention provides a method of producing an exogenous protein by use of the microorganism of the genus *Rhodococcus* having a high sensitivity to lysozyme.

[0009] The present inventors conducted studies with a view toward to attain an expression system for a recombinant protein by use of a microorganism of the genus Rhodococcus by overcoming a difficulty in extracting a cellular content. As a result, they found a novel microorganism of the genus Rhodococcus more sensitive to lysozyme at an extremely low concentration than a wild type strain. More specifically, mutation was induced in a wild type strain to obtain a mutant that cannot grow in a medium containing lysozyme. Mutation is usually induced by a chemical mutagen such as nitrosoguanidine or irradiation with radioactive rays. However, taking safety and convenience into consideration, ultraviolet ray irradiation is employed in the present invention. Furthermore, the present inventors found that cell lysis can be performed only by lysozyme treatment without pretreatment with penicillin or the like, and that a cellular content such as a recombinant protein accumulated in the cells can be extracted in a much easier manner than a conventional method. Based on these findings, the present invention was completed.

[0010] More specifically, the present invention includes

[0011] (1) A mutant of a microorganism of the genus *Rhodococcus* having a higher sensitivity to lysozyme than a wild-type microorganism of the genus *Rhodococcus*.

[0012] (2) The microorganism of the genus *Rhodococcus* according to item (1), in which the microorganism of the genus *Rhodococcus* is *Rhodococcus* erythropolis.

[0013] (3) The microorganism of the genus *Rhodococcus* according to item (2), in which the *Rhodococcus erythropolis* is *Rhodococcus erythropolis* strain L-65 (deposited on Jun. 12, 2002, originally at the International Patent Organism Depositary of the National Institute of Advanced Industrial Science and Technology (Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan) under Accession No. FERM BP-8443) or *Rhodococcus erythropolis* strain L-88 (deposited on Jun. 12, 2002, originally at the International Patent Organism Depositary of the National Institute of Advanced Industrial Science and Technology (Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan) under Accession No. FERM BP-8443) or *Rhodococcus erythropolis* strain L-88 (deposited on Jun. 12, 2002, originally at the International Patent Organism Depositary of the National Institute of Advanced Industrial Science and Technology (Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan, Accession No. FERM BP-8444).

[0014] (4) A method of producing a protein comprising

[0015] transforming a mutant of a microorganism of the genus *Rhodococcus* having a higher sensitivity to lysozyme than a wild-type microorganism of the genus *Rhodococcus* by a gene encoding an exogenous protein; expressing the gene; and treating the microorganism of the genus *Rhodococcus* with lysozyme, thereby extracting and recovering the protein.

[0016] (5) The method of producing a protein according to item (4), in which the microorganism of the genus *Rhodococcus* is *Rhodococcus erythropolis*.

[0017] (6) The method of producing a protein according to item (5), in which the *Rhodococcus erythropolis* is *Rhodococcus erythropolis* strain L-65 (Accession No. FERM BP-8443) or *Rhodococcus erythropolis* strain L-88 (Accession No. FERM BP-8444).

[0018] Now, the present invention will be explained in detail.

[0019] A microorganism of the genus Rhodococcus according to the present invention is a mutant microorganism of the genus Rhodococcus, which has a higher sensitivity to lysozyme than a wild-type microorganism of the genus Rhodococcus. The microorganism of the genus Rhodococcus is not limited to a specific species and includes Rhodococcus erythropolis, Rhodococcus fascians, and Rhodococcus opacus. The wild-type microorganism of the genus Rhodococcus refers to a microorganism belonging to the genus Rhodococcus and having no genetic mutation, for example, Rhodococcus erythropolis strain JCM 3201. More specifically, the microorganism of the genus Rhodococcus according to the present invention having a higher sensitivity to lysozyme is a mutant derived from a wild-type microorganism of the genus Rhodococcus as a parent strain, and having an increased sensitivity to lysozyme compared to the parent strain. The phrase "having an increased sensitivity to lysozyme" means that cell lysis may occur at a low lysozyme concentration. If cell growth is inhibited when lysozyme is added to a medium where a microorganism is cultured, it is said that the microorganism has a sensitivity to lysozyme. The sensitivity to lysozyme can be expressed by a minimum lysozyme concentration capable of inhibiting the growth of a microorganism (a minimum growth inhibitory lysozyme concentration). A source providing lysozyme is not limited, for example, egg-white lysozyme may be used. The minimum growth inhibitory lysozyme concentration is obtained as follows: for example, a microorganism of the genus Rhodococcus is prepared in a liquid medium at a density of 1×10 to 1×10^5 cells/10 µl. Lysozyme is serially diluted from a concentration of several hundreds μ g/ml to several μ g/ml. Each of the serial dilutions is added to 10 μ l of the liquid medium prepared above. The microorganism is cultured for several days. The lysozyme concentration that inhibits the growth of a microorganism of the genus Rhodococcus represents the minimum growth inhibitory lysozyme concentration. Alternatively, the degree of sensitivity to lysozyme can be determined by adding lysozyme to a predetermined concentration of a microorganism of the genus Rhodococcus, and monitoring a change in absorbence while culturing. In this case, a strain non-sensitive to lysozyme continues to grow without causing cell lysis by lysozyme, and thus absorbence increases with time, whereas a strain sensitive to lysozyme causes cell lysis by lysozyme, and absorbence decreases rapidly.

[0020] The minimum growth inhibitory lysozyme concentration of a microorganism of the genus *Rhodococcus* having a high sensitivity to lysozyme according to the present invention is preferably 50 μ g/ml or less, more preferably, 25 μ g/ml or less, and most preferably 13 μ g/ml or less. This is equal to or less than $\frac{1}{8}$, preferably $\frac{1}{16}$, and particularly preferably, $\frac{1}{30}$ of the minimum growth inhibitory lysozyme concentration of a wild type, that is, a parent strain.

[0021] A microorganism of the genus *Rhodococcus* generally has a high resistance to lysozyme, so that the microorganism cannot be lysed with lysozyme alone. Therefore, an antibiotic such as penicillin must be used at a high concentration to inhibit the cell wall synthesis of the microorganism during growth to weaken the cell wall, and then lysozyme is applied to the microorganism. However, the organism of the genus *Rhodococcus* according to the present invention can be lysed with lysozyme alone.

[0022] The organism of the genus *Rhodococcus* having a high sensitivity to lysozyme according to the present invention can be obtained by treating a wild-type microorganism of the genus Rhodococcus such as Rhodococcus erythropolis strain JCM 3201 with a chemical mutagen or a physical mutagen, culturing it in an agar medium, transferring the colonies thus grown onto a medium containing lysozyme and a medium not containing lysozyme, culturing both, and selecting a bacterial cell not grown in the medium containing lysozyme. The sensitivity to lysozyme can be determined by the aforementioned sensitivity test. Examples of such chemical mutagens include alkylation agents such as N-methyl-N'-nitro-N-nitrosoguanidine and mustard gas, non-alkylation agents such as hydradine and nitrite, DNA nucleotide analogs such as 5-bromo uracil, 2-aminopterin, and DNA intercalators, such as acrydine orange. Examples of such physical mutagens include ultraviolet rays, X-rays, y-rays, and neutron beam. A method of treating a microorganism with a mutagen, the concentration of a chemical mutagen to be used, and the intensity of a physical mutagen to be used may be appropriately selected in accordance with a known method.

[0023] Examples of such an microorganism of the genus *Rhodococcus* having a high sensitivity to lysozyme according to the present invention include *Rhodococcus erythropolis* strain L-65 (Accession No. FERM BP-8443) and *Rhodococcus erythropolis* strain L-88 (Accession No. FERM BP-8444).

3

[0024] In a microorganism of the genus *Rhodococcus* having a high sensitivity to lysozyme according to the present invention, its sensitivity to lysozyme is higher than that of a wild-type strain, that is, a parent stain; however, the sensitivity to at least one member of antibiotics such as ampicillin, kanamycin, chloramphenicol, tetracycline, and thiostreptone is equal to that of the wild strain and thus has no significant difference. Even if the sensitivity differs between them, the difference from the wild type is not so large as that from lysozyme sensitivity. More specifically, a microorganism of the genus Rhodococcus having a high sensitivity to the lysozyme according to the present invention has a resistant gene to a certain antibiotic integrated as a selection marker. Therefore, when the microorganism of the present invention is transformed by an expression vector having an exogenous gene integrated therein and a transformant is selected based on the selection marker, nontransformed microorganism of the genus Rhodococcus cannot grow since the sensitivity to the antibiotic is not lowered and thus only transformant can be selected. In this respect, as long as a microorganism of the genus Rhodococcus having a high sensitivity to lysozyme according to the present invention has a sensitivity to the antibiotic to be used for selection even if the sensitivity to other antibiotics is low, it can be used.

[0025] A recombinant protein can be efficiently obtained by using a microorganism of the genus Rhodococcus having a high sensitivity to lysozyme according to the present invention. To explain more specifically, the protein can be obtained by transforming a microorganism of the genus Rhodococcus having a high sensitivity to lysozyme according to the present invention with a gene encoding an exogenous protein derived from other species, culturing the transformed Rhodococcus microorganism in the conditions where the gene can be expressed, thereby expressing the exogenous protein, treating the microorganism having the expressed protein therein with lysozyme, thereby extracting the protein, and purifying and recovering the protein from the extract solution. The transformation of a microorganism of the genus *Rhodococcus* of the present invention may be performed in accordance with a known method. At this time, a transformation efficiency of a microorganism of the genus Rhodococcus with a reduced sensitivity to lysozyme is equivalent to that of a wild type strain, that is, a parent strain, even if there is a difference between them, the difference is not so significant. In some cases, the transformation efficiency is more or less lowered by the effect of introduction of mutation; however, there is no case where the efficiency of expressing and producing an exogenous protein is significantly reduced.

[0026] Transformation can be performed by using a known expression vector for a microorganism of the genus *Rhodococcus* or by expression vector pHN170 constructed by the present inventors such that the expression of the vector can be induced by thiostreptone.

[0027] A microorganism of the genus *Rhodococcus* transformed by integrating an exogenous gene therein is cultured. After the exogenous gene is expressed, the cells of the microorganism are collected by centrifugation, or the like, suspended in a buffer solution, such as a phosphate buffer, having lysozyme dissolved therein, and incubated at a temperature near an optimal temperature of lysozyme for several tens to several hours. The cells of the microorganism

are lysed by the action of lysozyme and the expressed protein is extracted into the buffer solution. The extracted protein is purified by a known protein purification method to obtain the protein. The concentration of lysozyme to be used in cell lysis is 0.1 mg/ml to 10 mg/ml, preferably about 1 mg/ml. Purification can be performed by use of any separation and purification method. For example, ammonium sulfate precipitation, gel filtration, ion exchange chromatography, and affinity chromatography may be used singly or in an appropriate combination. In the case where an expression product is present in the form of a fusion protein with GST, His tag, it may be purified by use of the nature of a peptide or a protein that is fused to a desired protein. To explain more specifically, since GST has an affinity for glutathione, the desired protein can be efficiently purified by affinity chromatography by use of a column having a carrier to which glutathione is attached.

[0028] The specification includes the specification and/or contents of the drawings of JP Patent Application No. 2002-239554 based on which the present application claims the priority.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] FIG. 1 shows photographs of LB agar mediums onto which serially diluted culture solutions are spotted for comparing their growth;

[0030] FIG. 2 is a graph showing a growth curve of *Rhodococcus erythropolis* strain L-65;

[0031] FIG. 3 is a graph showing a growth curve of *Rhodococcus erythropolis* strain L-88;

[0032] FIG. 4 is a graph showing a growth curve of *Rhodococcus erythropolis* strain JCM 3201; and

[0033] FIG. 5 shows SDS polyacrylamide electrophoresis of the cases where a PIP protein was expressed by *Rhodococcus erythropolis* strain L-65, L-88 and JCM3201.

BEST MODE FOR CARRYING OUT THE INVENTION

[0034] The present invention will be now explained with reference to Examples, which should not be construed as limiting the present invention.

EXAMPLE 1

[0035] Production of Lysozyme-Sensitive Bacterial Strain

[0036] Rhodococcus erythropolis strain JCM 3201 was cultured in LB medium (1% Difco Bacto Tryptone, 0.5% Difco Yeast Extract, and 1% sodium chloride) with shaking at 30° C. The LB medium was taken in the middle of the logarithmic growth period and appropriately diluted. The dilution was applied onto an LB medium plate containing 1.5% agar at a density of about 5×10^3 bacterial cells per plate, and the application surface was irradiated with 254 nm ultraviolet ray by means of an ultraviolet-ray irradiation apparatus (manufactured by Atto, power: 4 W) placed at a distance of 15 cm from the application surface for 20 seconds. The medium irradiated with the ultraviolet ray was cultured stand still at 30° C. for 2 days to obtain about 5×10^2 colonies per plate. The colonies were scraped by a cocktail stick and inoculated onto a 96-well plate filled with about 150 µl of LB medium. After the colonies were sufficiently

Jul. 27, 2006

suspended, a part of the suspension was inoculated onto a 96-well plate filled with 150 µl of LB medium containing lysozyme derived from egg-white in a concentration of 50 µg/ml (manufactured by Sigma, hereinafter simply referred to as "lysozyme"). The couple of plates thus obtained were cultured stand still at 30° C. for 2 days. As a result, a mutant strain capable of growing only in lysozyme-free LB medium was obtained as a lysozyme sensitive strain. Examples of such a novel lysozyme sensitive microorganism according to the present invention include Rhodococcus erythropolis strain L-65 and Rhodococcus erythropolis strain L-88, which was originally deposited on Jun. 12, 2002 under Accession Nos. FERM BP-8443 and FERM BP-8444, respectively at the International Patent Organism Depositary of the National Institute of Advanced Industrial Science and Technology (Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, Japan). A request for transferring the microorganisms from the original deposition to the international deposition based on the Budapest Treaty was made and accepted as of Jul. 28, 2003. The bacterial strain was inoculated in LB medium and cultured with shaking at 30° C. A part of the culture solution was taken in the middle of the logarithmic growth period and diluted with fresh LB medium so as to contain 1×10^5 , 1×10^4 , 1×10^3 , 1×10^2 , and 1×10 cells in 10 µl of the LB medium. The diluted culture solutions thus prepared were spotted onto each of LB agar mediums containing lysozyme in concentrations of 50, 25, 12.5 and $6.3 \mu g/ml$. After the mediums were cultured at 30° C. for 2 days, the presence or absence of bacterial cells grown on mediums was checked. In this manner, the minimum growth inhibition concentration was determined (Table 1 and FIG. 1). As shown in the figure, the culture solutions of bacterial strains JCM3201, L-65, and L-88 were dropped onto an LB agar medium containing no lysozyme and an LB agar medium containing lysozyme (12.5 µg/ml) and then subjected to culturing. The bacterial strains JCM3201, L-65, and L-88 cells were dropped respectively onto the upper stage, the middle stage, and the lower stage of the LB agar medium and cultured as shown in FIG. 1. The numbers of bacterial cells contained in culture solutions were 1×10^5 , 1×10^4 , 1×10^3 , 1×10^2 , and 1×10 cells in this order from the

TABLE 1

left.

Bacterial strain	Deposition No.	Minimum growth- inhibiting lysozyme concentration (µg/ml)
Rhodococcus erythropolis strain L-65	FERM BP-8443	12.5
Rhodococcus erythropolis strain L-88	FERM BP-8444	12.5
Rhodococcus erythropolis strain JCM 3201	ATCC25544	>400

${\rm EXAMPLE}\,\,2$

[0037] Turbidity change of *Rhodococcus erythropolis* strain L-65 culture solution by addition of lysozyme

[0038] To 100 ml of LB medium, *Rhodococcus erythropolis* strain L-65 was inoculated and cultured with shaking at 30° C. The absorbence (OD_{600}) of the culture solution was measured at an absorption wavelength of 600 nm every hour from the beginning of the logarithmic growth period. When

 OD_{600} reached about 0.2, the volume of the culture solution was divided into halves. To one of them, lysozyme was added to a final concentration of 12.5 µg/ml. No lysozyme was added to the other. While both solutions were further cultured continuously, the absorbence was measured. The results are shown in **FIG. 2**. The growth profiles of *Rhodococcus erythropolis* strain L-65 culture solutions with Lysozyme (12.5 µg/ml) and without lysozyme were shown by absorbence at 600 nm. When OD_{600} reached about 0.2, lysozyme was added (Indicated by the arrow in the figure). When lysozyme was added, a sharp decrease in absorbence was observed. This is considered because bacterial cell lysis was caused by lysozyme.

EXAMPLE 3

[0039] Turbidity change of *Rhodococcus erythropolis* strain L-88 culture solution by addition of lysozyme

[0040] The same operation as in Example 2 was performed by use of *Rhodococcus erythropolis* strain L-88. Absorbence was measured and the results are shown in **FIG. 3**. The growth profiles of culture solutions of *Rhodococcus erythropolis* strain L-88 with lysozyme (12.5 μ g/ml) and without lysozyme were shown by absorbence at 600 nm. When OD₆₀₀ reached about 0.2, lysozyme was added (Indicated by the arrow in the figure). When lysozyme was added, a sharp decrease in absorbence was observed. This is considered because bacterial cell lysis was caused by lysozyme.

COMPARATIVE EXAMPLE 1

[0041] Turbidity change of *Rhodococcus erythropolis* strain JCM 3201 culture solution by addition of lysozyme

[0042] The same operation as in Example 2 was performed by use of *Rhodococcus erythropolis* strain JCM 3201. Absorbence was measured and the results are shown in **FIG. 4**. The growth profiles of culture solutions of *Rhodococcus erythropolis* strain JCM 3201 with lysozyme (12.5 μ g/ml) and without lysozyme were shown by absorbence at 600 nm. When OD₆₀₀ reached about 0.2, lysozyme was added (Indicated by the arrow in the figure). Regardless of the presence or absence of lysozyme, the same tendency of growth was observed.

EXAMPLE 4

[0043] Sensitivity of lysozyme sensitive bacterial strain to ampicillin

[0044] The sensitivity of *Rhodococcus ervthropolis* strain L-65 and L-88 to ampicillin was determined in the same manner as in Example 1. To explain more specifically, the bacterial cells of each strain were inoculated in LB medium and cultured with shaking at 30° C. A part of the culture solution was taken in the middle of logarithmic growth period and diluted with fresh LB medium so as to contain 1×10^5 , 1×10^4 , 1×10^3 , 1×10^2 , and 1×10 cells in 10 µl of the LB medium. The diluted culture solutions thus prepared were dropped onto each of LB agar mediums containing lysozyme in concentrations of 15, 10, 1, and 0.1 µg/ml. After the mediums were cultured at 30° C. for 2 days and the presence or absence of bacterial cells grown on the mediums was checked. In this manner, the minimum growth inhibition concentration was determined (Table 2). Similarly, the wild type and the mutant strain were compared for sensitivity to

TABLE 2

Bacterial strain	Deposition No.	Minimum growth- inhibiting ampicillin concentration (μg/ml)
Rhodococcus erythropolis strain L-65	FERM BP-8443	1
Rhodococcus erythropolis strain L-88	FERM BP-8444	1
Rhodococcus erythropolis strain JCM 3201	ATCC25544	15

EXAMPLE 5

[0045] Transformation efficiency of lysozyme sensitive strain

[0046] The transformation of *Rhodococcus erythropolis* was performed by an electroporation method. The method will be described in detail below. Rhodococcus erythropolis strain JCM 3201, L-65, and L-88 were cultured in 100 ml of LB medium with shaking at 30° C. until they reach their logarithmic growth periods. The culture solutions were cooled on ice for 30 minutes, and centrifugally separated to recover cells. To the recovered cells, 100 ml of ice-cooled sterilized water was added, stirred well, and again centrifugally separated to recover cells. To the recovered cells, 100 ml of an ice-cooled 10% glycerin solution was added, stirred well, and centrifugally separated to recover cells. The cells were washed again with the ice-cooled 10% glycerin solution and suspended in 5 ml of an ice-cold 10% glycerin solution. Then, 400 µl of the resultant cells were mixed with plasmid DNA (pHN144; Nakashima and Tamura; the full length sequence is represented by SEQ ID No: 1) capable of self-replicating in Rhodococcus erythropolis. The mixture solution was transferred to an electroporation cuvette (0.2 cm gap cuvette manufactured by Bio-Rad) and applied with an electric pulse by a gene introduction apparatus, gene pulser II, (manufactured by Bio-Rad) at an electric field of 12.5 kV/cm in strength, a capacitance of 25 μ F (the pulse controller), and an external resistance of 400 Ω . The mixture of cells and DNA treated with the electric pulse was mixed with 1 ml of LB medium and cultured at 30° C. for 4 hours. Thereafter, cells were collected and applied onto LB agar medium containing thiostreptone in a concentration of 10 µg/ml and cultured at 30° C. for 3 days to obtain transformants for each case. The transformation efficiency (the number of colonies formed) per 1 µg of DNA is shown in Table 3.

TABLE 3

Bacterial strain	Deposition No.	Transformation efficiency rate
Rhodococcus erythropolis strain L-65	FERM BP-8443	2.6×10^{5}
Rhodococcus erythropolis strain L-88	FERM BP-8444	2.5×10^5
Rhodococcus erythropolis strain JCM 3201	ATCC25544	4.0×10^{5}

EXAMPLE 6

[0047] Extraction of recombinant protein produced by *Rhodococcus erythropolis* strain L-65

[0048] A plasmid (pHN170, Nakashima and Tamura: the full length sequence is represented by SEQ ID No: 2) was constructed such that it could self-replicate in a bacterial cell of Rhodococcus ervthropolis and could be induced by thiostreptone to express a proline iminopeptidase (hereinafter referred to as "PIP") protein (T. Tamura et al., FEBS Lett. 398, 101-105 (1996)) having a 6× histidine tag at the C terminal. This plasmid was introduced into Rhodococcus erythropolis strain L-65 by an electroporation method. Transformants were screened on LB agar medium containing tetracycline (20 µg/ml). The transformants were inoculated on 4 ml of LB medium containing tetracycline (8 μ g/ml) and cultured with shaking at 30° C. until the absorbence at an absorption wavelength of 600 nm reached 0.8. The entire culture solution was added to 40 ml of LB medium containing thiostreptone (1 µg/ml) and cultured with rotation in a vaned flask for 16 hours. After PIP protein was induced to express, bacterial cells were centrifugally collected at 1,500×g for 15 minutes. After the cells thus collected was suspended in 4 ml of a 50 mM phosphate buffer (pH8.0) containing 300 mM salt, lysozyme was added so as to obtain a final concentration of 1 mg/ml. The resultant solution was incubated at 37° C. for one hour, cooled on ice, and centrifuged at 10,000×g for 15 minutes to separate the supernatant (s) and the precipitate (p). An aliquot of 1 ml was taken from the obtained supernatant (s) and placed in another microcentrifuge tube. To the microcentrifuge tube, 50 µl of Ni-NTA Superflow (manufactured by QIAGEN) was added, which had been previously equilibrated with a 50 mM phosphate buffer (pH 8.0) containing 300 mM salt. The resultant mixture was incubated at 4° C. for one hour while turning it upside down, washed three times with 1 ml of a 50 mM phosphate buffer (pH 6.0) containing 300 mM salt and 10% glycerin, and thereafter, eluted with 50 µl of a 50 mM phosphate buffer (pH 6.0) containing 500 mM EDTA, 300 mM salt and 10% glycerin to obtain 6× histidine-fused PIP protein. An aliquot (10 µl) was taken from the protein thus obtained and subjected to SDS polyacrylamide gel electrophoresis. As a result, a clear band was detected near the molecular weight (34.3 KDa), which was predicted from the amino acid sequence of the $6 \times$ histidine-fused PIP protein (FIG. 5). On the other hand, the precipitate (p) was resuspended in 1 ml of a 100 mM sodium dihydrogen phosphate -10 mM tris chloride buffer (pH 8.0) containing 8M urea, allowed to stand alone for 30 minutes, and subjected to centrifugation at 10,000×g for 15 minutes. The resultant supernatant was transferred to a new microcentrifuge tube, 50 µl of Ni-NTA Superflow, which had been previously equilibrated with a 100 mM sodium dihydrogen phosphate -10 mM tris chloride buffer (pH 8.0) containing 8M urea, was added to the microcentrifuge tube, and incubated at room temperature for one hour while turning upside down. After the microcentrifuge tube was washed three times with 1 ml of a 100 mM sodium dihydrogen phosphate -10 mM tris chloride buffer (pH 6.3) containing 8M urea, it was subjected to elution with 50 µl of a 100 mM sodium dihydrogen phosphate -10 mM tris chloride buffer (pH 8.0) containing 500 mM EDTA and 8M urea to obtain 6× histidine-fused PIP protein. An aliquot (10 µl) was taken from the protein thus obtained and subjected to SDS polyacrylamide gel electrophoresis (FIG. 5). Reference symbol

M represents a molecular marker and molecular weight is shown at the left side of the figure to indicate an approximate molecular weight of each band. Band patterns of individual lanes are shown in **FIG. 5** as follows.

[0049] Lane 1 (JCM 3201,s) shows an electrophoretic pattern of a sample obtained from the supernatant in the case where PIP is expressed by bacterial strain JCM 3201. Since cell lysis rarely takes place in a buffer solution under nondenaturation condition (containing no urea), a desired band (indicated by the arrow) is not detected. Lane 2 (JSM3201, p) shows an electrophoretic pattern of a sample obtained from the precipitate in the case where PIP is expressed by bacterial strain JSM 3201. Lysis takes place slightly in a buffer solution under denaturation conditions (containing urea) and thus a thin band of interest is detected.

[0050] Lane 3 (JCM3201+amp,s) shows an electrophoretic pattern of a sample obtained from the supernatant in the case where PIP is expressed by bacterial strain JCM 3201 where the sample is treated with ampicillin for 2 hours before cell collection. Since the sensitivity to lysozyme is increased by the treatment with ampicillin, cell lysis takes place even in nondenaturation conditions. As a result, a desired band can be clearly confirmed.

[0051] Lane 4 (JSM3201+amp, p) shows an electrophoretic pattern of a sample obtained from the precipitate in the case where PIP is expressed by bacterial strain JCM 3201 where the sample is treated with ampicillin for 2 hours before cell collection. It is considered that even though the cells previously treated with ampicillin do not cause cell lysis in a buffer under the nondenaturation conditions, they lyse in a buffer under denaturation conditions to give a detectable desired band.

[0052] Lane 5 (L-65, s) shows an electrophoretic pattern of a sample obtained from the supernatant in the case where PIP is expressed by bacterial strain L-65. Cells were lysed completely with lysozyme treatment to give a detectable desired band.

[0053] Lane 6 (L-65, p) shows an electrophoretic pattern of a sample obtained from the precipitate in the case where PIP is expressed by bacterial strain L-65. Since the precipitate is considered to contains only residual cells after lysis, the desired band is not detected.

[0054] Lane 7 (L-88, s) shows the electrophoretic pattern of a sample obtained from the supernatant in the case where PIP is expressed by bacterial strain L-88. The same phenomenon as in the case of bacterial stain L-65 is considered.

[0055] Lane 8 (L-88, p) shows the electrophoretic pattern of a sample obtained from the precipitate in the case where PIP is expressed by bacterial strain L-88. The same phenomenon as in the case of stain L-65 is considered.

[0056] As for the antibiotic used in the aforementioned operation, a required amount of a solution containing 5 mg

of tetracycline dissolved in 1 ml of 50 wt % ethanol or 10 mg of thiostreptone dissolved in 1 ml of dimethylsulfoxide was used.

EXAMPLE 7

[0057] Extraction of recombinant protein produced by *Rhodococcus erythropolis* strain L-88

[0058] The same operation as in Example 6 was performed except that *Rhodococcus erythropolis* strain L-88 was used in place of *Rhodococcus erythropolis* strain L-65 (FIG. 5).

COMPARATIVE EXAMPLE 2

[0059] Extraction of recombinant protein produced by *Rhodococcus erythropolis* strain JCM 3201

[0060] The same operation as in Example 6 was performed except that *Rhodococcus erythropolis* strain JCM 3201 was used in place of *Rhodococcus erythropolis* strain L-65 (FIG. 5).

COMPARATIVE EXAMPLE 3

[0061] Extraction of recombinant protein produced by *Rhodococcus erythropolis* strain JCM 3201

[0062] A transformant was prepared in the same manner as in Example 6 except that *Rhodococcus erythropolis* strain JCM 3201 was used in place of *Rhodococcus erythropolis* strain L-65. The expression of PIP protein was induced by thiostreptone. Two hours before cell collection, 480 μ l of an aqueous solution of ampicillin (50 mg/ml) was added (a final concentration of 600 μ g/ml) and subjected to cell collection. Thereafter, the same operations as in Example 6 were performed and the obtained sample was subjected to electrophoresis (FIG. 5).

[0063] All publications, patents and patent applications cited herein are incorporated herein in its entirety by reference.

INDUSTRIAL APPLICABILITY

[0064] As shown in Examples, a microorganism of the genus *Rhodococcus* of the present invention has an increased sensitivity to lysozyme compared to a wild type strain. The transformation efficiency of the microorganism of the present invention is not significantly changed from that of the wild-type stain. It is therefore possible to efficiently transform a microorganism of the genus *Rhodococcus* of the present invention by a gene encoding the exogenous protein, express the exogenous protein, cause cell lysis with lysozyme, and extract and recover the protein easily.

Sequence listing free text

[0065] SEQ ID No. 1: Plasmid pHN 144

[0066] SEQ ID No. 2: Plasmid pHN 170

<210> SEQ ID NO 1 <211> LENGTH: 5108 <212> TYPE: DNA

SEQUENCE LISTING

<pre></pre> <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: Plasmid pHN144						
<400> SEQUENCE: 1						
gagetegace gegegggtee eg	ygacgggga agagcgggga	gctttgccag agagcgacga	60			
cttccccttg cgttggtgat tg	geeggteag ggeageeate	cgccatcgtc gcgtagggtg	120			
tcacacccca ggaatcgcgt ca	actgaacac agcagccggt	aggacgacca tgactgagtt	180			
ggacaccatc gcaaatccgt cc	cgatecege ggtgeagegg	atcatcgatg tcaccaagcc	240			
gtcacgatcc aacataaaga ca	aacgttgat cgaggacgtc	gagcccctca tgcacagcat	300			
cgcggccggg gtggagttca to	cgaggtcta cggcagcgac	agcagtcctt ttccatctga	360			
gttgctggat ctgtgcgggc gg	gcagaacat accggtccgc	ctcatcgact cctcgatcgt	420			
caaccagttg ttcaagggggg ag	geggaagge caagacatte	ggcatcgccc gcgtccctcg	480			
cccggccagg ttcggcgata to	cgcgagccg gcgtggggac	gtcgtcgttc tcgacggggt	540			
gaagatcgtc gggaacatcg gc	cgcgatagt acgcacgtcg	ctcgcgctcg gagcgtcggg	600			
gatcatcctg gtggacagtg ac	catcaccag catcgcggac	cggcgtctcc aaagggccag	660			
ccgaggttac gtcttctccc tt	tecegtegt teteteeggt	cgcgaggagg ccatcgcctt	720			
cattcgggac agcggtatgc ag	gctgatgac gctcaaggcg	gatggcgaca tttccgtgaa	780			
ggaactcggg gacaatccgg at	teggetgge ettgetgtte	ggcagcgaaa agggtgggcc	840			
ttccgacctg ttcgaggagg cg	gtetteege eteggtttee	atccccatga tgagccagac	900			
cgagtetete aacgttteeg tt	tteeetegg aategegetg	cacgagagga tcgacaggaa	960			
tctcgcggcc aaccgataag cg	geetetgtt eeteggaege	tcggttcctc gacctcgatt	1020			
cgtcagtgat gatcacctca ca	acggcagcg atcaccactg	acatatcgag gtcaacggtc	1080			
gtggtccggg cgggcactcc to	cgaaggcgc ggccgacgcc	cttgaacgac tcgatgactc	1140			
tagaggatcc ccgggtaccg ag	gctcgtcag gtggcacttt	tcggggaaat gtgcgcggaa	1200			
cccctatttg tttatttttc ta	aaatacatt caaatatgta	tccgctcatg agacaataac	1260			
cctgataaat gcttcaataa ta	attgaaaaa ggaagagtat	gagtattcaa catttccgtg	1320			
tegeeettat teeettttt ge	eggeatttt geetteetgt	ttttgctcac ccagaaacgc	1380			
tggtgaaagt aaaagatgct ga	aagatcagt tgggtgcacg	agtgggttac atcgaactgg	1440			
atctcaacag cggtaagatc ct	ttgagagtt ttcgccccga	agaacgtttt ccaatgatga	1500			
gcacttttaa agttctgcta tg	gtggcgcgg tattatcccg	tattgacgcc gggcaagagc	1560			
aactcggtcg ccgcatacac ta	atteteaga atgaettggt	tgagtactca ccagtcacag	1620			
aaaagcatct tacggatggc at	tgacagtaa gagaattatg	cagtgctgcc ataaccatga	1680			
gtgataacac tgcggccaac tt	tacttctga caacgatcgg	aggaccgaag gagctaaccg	1740			
cttttttgca caacatgggg ga	atcatgtaa ctcgccttga	tcgttgggaa ccggagctga	1800			
atgaagccat accaaacgac ga	agcgtgaca ccacgatgcc	tgtagcaatg gcaacaacgt	1860			
tgcgcaaact attaactggc ga	aactactta ctctagcttc	ccggcaacaa ttaatagact	1920			
ggatggaggc ggataaagtt gc	caggaccac ttctgcgctc	ggcccttccg gctggctggt	1980			
ttattgctga taaatctgga gc	ccggtgagc gtgggtctcg	cggtatcatt gcagcactgg	2040			
ggccagatgg taagccctcc cg	gtatcgtag ttatctacac	gacggggagt caggcaacta	2100			

tggatgaacg	aaatagacag	atcgctgaga	taggtgcctc	actgattaag	cattggtaac	2160
tgtcagacca	agtttactca	tatatacttt	agattgattt	aaaacttcat	ttttaattta	2220
aaaggatcta	ggtgaagatc	ctttttgata	atctcatgac	caaaatccct	taacgtgagt	2280
tttcgttcca	ctgagcgtca	gaccccgtag	aaaagatcaa	aggatcttct	tgagatcctt	2340
ttttctgcg	cgtaatctgc	tgcttgcaaa	caaaaaaacc	accgctacca	gcggtggttt	2400
gtttgccgga	tcaagagcta	ccaactcttt	ttccgaaggt	aactggcttc	agcagagcgc	2460
agataccaaa	tactgttctt	ctagtgtagc	cgtagttagg	ccaccacttc	aagaactctg	2520
tagcaccgcc	tacatacctc	gctctgctaa	tcctgttacc	agtggctgct	gccagtggcg	2580
ataagtcgtg	tcttaccggg	ttggactcaa	gacgatagtt	accggataag	gcgcagcggt	2640
cgggctgaac	ggggggttcg	tgcacacagc	ccagcttgga	gcgaacgacc	tacaccgaac	2700
tgagatacct	acagcgtgag	ctatgagaaa	gcgccacgct	tcccgaaggg	agaaaggcgg	2760
acaggtatcc	ggtaagcggc	agggtcggaa	caggagagcg	cacgagggag	cttccagggg	2820
gaaacgcctg	gtatctttat	agtcctgtcg	ggtttcgcca	cctctgactt	gagcgtcgat	2880
ttttgtgatg	ctcgtcaggg	gggcggagcc	tatggaaaaa	cgccagcaac	gcggcctttt	2940
tacggttcct	ggccttttgc	tggccttttg	ctcacatgtt	ctttcctgcg	ttatcccctg	3000
attctgtgga	taaccgtatt	accgcctttg	agtgagctga	taccgctcgc	cgcagccgaa	3060
cgaccgagcg	cagcgagtca	gtgagcgagg	aagcggaaga	gcgcccaata	cgcaaaccgc	3120
ctctccccgc	gcgttggccg	attcattaat	gcagctggca	cgactagttg	tacacccgag	3180
aagctcccag	cgtcctcctg	ggccgcgata	ctcgaccacc	acgcacgcac	accgcactaa	3240
cgattcggcc	ggcgctcgat	tcggccggcg	ctcgattcgg	ccggcgctcg	attcggccgg	3300
cgctcgattc	ggccggcgct	cgattcggcc	gagcagaaga	gtgaacaacc	accgaccacg	3360
cttccgctct	gcgcgccgta	cccgacctac	ctcccgcagc	tcgaagcagc	tcccgggagt	3420
accgccgtac	tcacccgcct	gtgctcacca	tccaccgacg	caaagcccaa	cccgagcaca	3480
cctcttgcac	caaggtgccg	accgtggctt	tccgctcgca	gggttccaga	agaaatcgaa	3540
cgatccagcg	cggcaaggtt	caaaaagcag	gggttggtgg	ggaggaggtt	ttggggggtg	3600
tcgccgggat	acctgatatg	gctttgtttt	gcgtagtcga	ataattttcc	atatagcctc	3660
ggcgcgtcgg	actcgaatag	ttgatgtggg	cgggcacagt	tgccccatga	aatccgcaac	3720
ggggggcgtg	ctgagcgatc	ggcaatgggc	ggatgcggtg	ttgcttccgc	accggccgtt	3780
cgcgacgaac	aacctccaac	gaggtcagta	ccggatgagc	cgcgacgacg	cattggcaat	3840
gcggtacgtc	gagcattcac	cgcacgcgtt	gctcggatct	atcgtcatcg	actgcgatca	3900
cgttgacgcc	gcgatgcgcg	cattcgagca	accatccgac	catccggcgc	cgaactgggt	3960
tgcacaatcg	ccgtccggcc	gcgcacacat	cggatggtgg	ctcggcccca	accacgtgtg	4020
ccgcaccgac	agcgcccgac	tgacgccact	gcgctacgcc	caccgcatcg	aaaccggcct	4080
caagatcagc	gtcggcggcg	atttcgcgta	tggcgggcaa	ctgaccaaaa	acccgattca	4140
ccccgattgg	gagacgatct	acggcccggc	caccccgtac	acattgcggc	agctggccac	4200
catccacaca	ccccggcaga	tgccgcgtcg	gcccgatcgg	gccgtgggcc	tgggccgcaa	4260
cgtcaccatg	ttcgacgcca	cccggcgatg	ggcatacccg	cagtggtggc	aacaccgaaa	4320
cggaaccggc	cgcgactggg	accatctcgt	cctgcagcac	tgccacgccg	tcaacaccga	4380

gttcacgaca ccactgccgt tcaccgaagt acgcgccacc gcgcaatcca tctccaaatg 4440 gatetggege aattteaceg aagaacagta eegageeega caagegeate teggteaaaa 4500 aggeggeaag geaacgaeae tegecaaaea agaageegte egaaaeaatg eaagaaagta 4560 4620 cgacgaacat acgatgcgag aggcgattat ctgatgggcg gagccaaaaa tccggtgcgc cgaaagatga cggcagcagc agcagccgaa aaattcggtg cctccactcg cacaatccaa 4680 cgcttgtttg ctgagccgcg tgacgattac ctcggccgtg cgaaagctcg ccgtgacaaa 4740 gctgtcgagc tgcggaagca ggggttgaag taccgggaaa tcgccgaagc gatggaactc 4800 tcgaccggga tcgtcggccg attactgcac gacgcccgca ggcacggcga gatttcagcg 4860 gaggatetgt eggegtaace aagteagegg gttgtegggt teeggeegge geteggeaet 4920 cggaccggcc ggcggatggt gttctgcctc tggcgcagcg tcagctaccg ccgaaggcct 4980 gtcatcgacc ggcttcgact gaagtatgag caacgtcaca gcctgtgatt ggatgatccg 5040 ctcacgctcg accgctacct gttcagctgc cgcccgctgg gcatgagcaa cggccaactc 5100 5108 tcgttcaa <210> SEQ ID NO 2 <211> LENGTH: 8971 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: Plasmid pHN170 <400> SEQUENCE: 2 gagetegace gegegggtee eggaegggga agagegggga getttgeeag agagegaega 60 120 cttccccttg cgttggtgat tgccggtcag ggcagccatc cgccatcgtc gcgtagggtg 180 tcacacccca ggaatcgcgt cactgaacac agcagccggt aggacgacca tgactgagtt ggacaccatc gcaaatccgt ccgatcccgc ggtgcagcgg atcatcgatg tcaccaagcc 240 300 gtcacgatcc aacataaaga caacgttgat cgaggacgtc gagcccctca tgcacagcat 360 cgcggccggg gtggagttca tcgaggtcta cggcagcgac agcagtcctt ttccatctga gttgctggat ctgtgcgggc ggcagaacat accggtccgc ctcatcgact cctcgatcgt 420 caaccagttg ttcaaggggg agcggaaggc caagacattc ggcatcgccc gcgtccctcg 480 cccggccagg ttcggcgata tcgcgagccg gcgtggggac gtcgtcgttc tcgacggggt 540 gaagatcgtc gggaacatcg gcgcgatagt acgcacgtcg ctcgcgctcg gagcgtcggg 600 gatcatcctg gtggacagtg acatcaccag catcgcggac cggcgtctcc aaagggccag 660 ccgaggttac gtcttctccc ttcccgtcgt tctctccggt cgcgaggagg ccatcgcctt 720 780 cattcgggac agcggtatgc agctgatgac gctcaaggcg gatggcgaca tttccgtgaa 840 ggaactcggg gacaatccgg atcggctggc cttgctgttc ggcagcgaaa agggtgggcc 900 ttccgacctg ttcgaggagg cgtcttccgc ctcggtttcc atccccatga tgagccagac 960 cgagtetete aacgttteeg ttteeetegg aategegetg caegagagga tegacaggaa tctcgcggcc aaccgataag cgcctctgtt cctcggacgc tcggttcctc gacctcgatt 1020 cgtcagtgat gatcacctca cacggcagcg atcaccactg acatatcgag gtcaacggtc 1080

gtggtccggg cgggcactcc tcgaaggcgc ggccgacgcc cttgaacgac tcgatgactc

1140

9

				-contir	nued	
tagagtaacg	ggctactccg	tttaacggac	cccgttctca	cgctttaggc	ttgaccccgg	1200
agcctgcatg	gggcattccg	ccgtgaaccc	ggtggaatgc	ccccggcacc	cgggctttcc	1260
agcaaagatc	acctggcgcc	gatgagtaag	gcgtacagaa	ccactccaca	ggaggaccgt	1320
cgagatgaaa	tctaacaatg	cgctcatcgt	catcctcggc	accgtcaccc	tggatgctgt	1380
aggcataggc	ttggttatgc	cggtactgcc	gggcctcttg	cgggatatcg	tccattccga	1440
cagcatcgcc	agtcactatg	gcgtgctgct	agcgctatat	gcgttgatgc	aatttctatg	1500
cgcacccgtt	ctcggagcac	tgtccgaccg	ctttggccgc	cgcccagtcc	tgctcgcttc	1560
gctacttgga	gccactatcg	actacgcgat	catggcgacc	acacccgtcc	tgtggattct	1620
ctacgccgga	cgcatcgtgg	ccggcatcac	cggcgccaca	ggtgcggttg	ctggcgccta	1680
tatcgccgac	atcaccgatg	gggaagatcg	ggctcgccac	ttcgggctca	tgagcgcttg	1740
tttcggcgtg	ggtatggtgg	caggccccgt	ggccggggga	ctgttgggcg	ccatctcctt	1800
gcatgcacca	ttccttgcgg	cggcggtgct	caacggcctc	aacctactac	tgggctgctt	1860
cctaatgcag	gagtcgcata	agggagagcg	tcgtccgatg	cccttgagag	ccttcaaccc	1920
agtcagctcc	ttccggtggg	cgcgggggcat	gactatcgtc	gccgcactta	tgactgtctt	1980
ctttatcatg	caactcgtag	gacaggtgcc	ggcagcgctc	tgggtcattt	tcggcgagga	2040
ccgctttcgc	tggagcgcga	cgatgatcgg	cctgtcgctt	gcggtattcg	gaatcttgca	2100
cgccctcgct	caagccttcg	tcactggtcc	cgccaccaaa	cgtttcggcg	agaagcaggc	2160
cattatcgcc	ggcatggcgg	ccgacgcgct	gggctacgtc	ttgctggcgt	tcgcgacgcg	2220
aggctggatg	gccttcccca	ttatgattct	tctcgcttcc	ggcggcatcg	ggatgcccgc	2280
gttgcaggcc	atgctgtcca	ggcaggtaga	tgacgaccat	cagggacagc	ttcaaggatc	2340
gctcgcggct	cttaccagcc	taacttcgat	cattggaccg	ctgatcgtca	cggcgattta	2400
tgccgcctcg	gcgagcacat	ggaacgggtt	ggcatggatt	gtaggcgccg	ccctatacct	2460
tgtctgcctc	cccgcgttgc	gtcgcggtgc	atggagccgg	gccacctcga	cctgaatgga	2520
agccggcggc	acctcgctaa	cggattcacc	actccaagaa	ttggagccaa	tcaattcttg	2580
cggagaactg	tgaatgcgca	aaccaaccct	tggcagaaca	tatccatcgc	gtccgccatc	2640
tccagcagcc	gcacgcggcg	catctcgggc	agcgttgggt	cctggccacg	ggtgcgcaac	2700
tagaattgat	ctcctcgacc	gccaattggg	catctgagaa	tcatctgcgt	ttctcgcacg	2760
caacgtactt	gcaacgttgc	aactcctagt	gttgtgaatc	acaccccacc	aaaaaataaa	2820
attgcagtca	ccgatttggt	gggtgcgccc	aggaagatca	cgtttacata	ggagcttgca	2880
atgagctact	ccgtgggaca	ggtggccggc	ttcgccggag	tgacggtgcg	cacgctgcac	2940
cactacgacg	acatcggcct	gctcgtaccg	agcgagcgca	gccacgcggg	ccaccggcgc	3000
tacagcgacg	ccgacctcga	ccggctgcag	cagatcctgt	tctaccggga	gctgggcttc	3060
ccgctcgacg	aggtcgccgc	cctgctcgac	gacccggccg	cggacccgcg	cgcgcacctg	3120
cgccgccagc	acgagctgct	gtccgcccgg	atcgggaaac	tgcagaagat	ggcggcggcc	3180
gtggagcagg	cgatggaggc	acgcagcatg	ggaatcaacc	tcaccccgga	ggagaagttc	3240
gaggtcttcg	gcgacttcga	ccccgaccag	tacgaggagg	aggtccggga	acgctggggg	3300
aacaccgacg	cctaccgcca	gtccaaggag	aagaccgcct	cgtacaccaa	ggaggactgg	3360
cagcgcatcc	aggacgaggc	cgacgagctc	acccggcgct	tcgtcgccct	gatggacgcg	3420

-continued	
ggtgagcccg ccgactccga gggggcgatg gacgccgccg aggaccaccg gcagggcatc	3480
gcccgcaacc actacgactg cgggtacgag atgcacacct gcctgggcga gatgtacgtg	3540
teegaegaae gttteaegeg aaacategae geegeeaage egggeetege egeetaeatg	3600
cgcgacgcga tcctcgccaa cgccgtccgg cacaccccct gagcggtggt cgtggcccgg	3660
gtctcccgcc cggtctcacc ccacggctca ctcccgggcc acgaccaccg ccgtcccgta	3720
cgcgcacacc tcggtgccca cgtccgccgc ctccgtcacg tcgaaacgga agatccccgg	3780
gtaccgagct cgtcaggtgg cacttttcgg ggaaatgtgc gcggaacccc tatttgttta	3840
tttttctaaa tacattcaaa tatgtatccg ctcatgagac aataaccctg ataaatgctt	3900
caataatatt gaaaaaggaa gagtatgagt attcaacatt tccgtgtcgc ccttattccc	3960
ttttttgcgg cattttgcct tcctgttttt gctcacccag aaacgctggt gaaagtaaaa	4020
gatgctgaag atcagttggg tgcacgagtg ggttacatcg aactggatct caacagcggt	4080
aagateettg agagtttteg eeecgaagaa egtttteeaa tgatgageae ttttaaagtt	4140
ctgctatgtg gcgcggtatt atcccgtatt gacgccgggc aagagcaact cggtcgccgc	4200
atacactatt ctcagaatga cttggttgag tactcaccag tcacagaaaa gcatcttacg	4260
gatggcatga cagtaagaga attatgcagt gctgccataa ccatgagtga taacactgcg	4320
gccaacttac ttctgacaac gatcggagga ccgaaggagc taaccgcttt tttgcacaac	4380
atgggggatc atgtaactcg ccttgatcgt tgggaaccgg agctgaatga agccatacca	4440
aacgacgagc gtgacaccac gatgcctgta gcaatggcaa caacgttgcg caaactatta	4500
actggcgaac tacttactct agcttcccgg caacaattaa tagactggat ggaggcggat	4560
aaagttgcag gaccacttct gcgctcggcc cttccggctg gctggtttat tgctgataaa	4620
totggagoog gtgagogtgg gtotogoggt atcattgoag caotggggoo agatggtaag	4680
ccctcccgta tcgtagttat ctacacgacg gggagtcagg caactatgga tgaacgaaat	4740
agacagatcg ctgagatagg tgcctcactg attaagcatt ggtaactgtc agaccaagtt	4800
tactcatata tactttagat tgatttaaaa cttcattttt aatttaaaag gatctaggtg	4860
aagateettt ttgataatet eatgaceaaa ateeettaae gtgagtttte gtteeaetga	4920
gcgtcagacc ccgtagaaaa gatcaaagga tcttcttgag atccttttt tctgcgcgta	4980
atctgctgct tgcaaacaaa aaaaccaccg ctaccagcgg tggtttgttt gccggatcaa	5040
gagctaccaa ctctttttcc gaaggtaact ggcttcagca gagcgcagat accaaatact	5100
gttcttctag tgtagccgta gttaggccac cacttcaaga actctgtagc accgcctaca	5160
tacctcgctc tgctaatcct gttaccagtg gctgctgcca gtggcgataa gtcgtgtctt	5220
accgggttgg actcaagacg atagttaccg gataaggcgc agcggtcggg ctgaacgggg	5280
ggttcgtgca cacagcccag cttggagcga acgacctaca ccgaactgag atacctacag	5340
cgtgagctat gagaaagcgc cacgcttccc gaagggagaa aggcggacag gtatccggta	5400
agcggcaggg tcggaacagg agagcgcacg agggagcttc cagggggaaa cgcctggtat	5460
ctttatagtc ctgtcgggtt tcgccacctc tgacttgagc gtcgattttt gtgatgctcg	5520
tcaggggggc ggagcctatg gaaaaacgcc agcaacgcgg cctttttacg gttcctggcc	5580
ttttgctggc cttttgctca catgttcttt cctgcgttat cccctgattc tgtggataac	5640
cgtattaccg cctttgagtg agctgatacc gctcgccgca gccgaacgac cgagcgcagc	5700

11

gagtagtag gagagaag gagagagag gagagaag gagagagag			-continued		
segorgono grogingina caacegyg igyacgya orginizacy igityicogi 5880 gegortat acaactog igityityi agyattag agyattag agyityag acatyityi alayatig 6000 ataatyatya agyiytta gyatyaata oyaaayaaa tiyityita ottaagyat 6660 ataatyatya agyiytta gyatyaata oyaaayaaa tiyityita ottaagyat 6660 ataatyatya agyiytta gyatyaata oyaaayaaa tiyityita ottaagyat 6100 ataatyatya atattigaa atattiga agaatyaa gitaagyatyi gatyyaaa 6300 atayiytyi agtacotyg agoataco gataatya agtacogaa agyatyi gatyyaaa 6300 atayiytyi agtacotyg agoataa oottayataa agtacogaa agyatyi gatyyaaa 6300 atayiytii agtacotyg gagoata oottayataa agtacogaa agyatyi gatyyaaa 6300 atayiytii agtacotyg gagoata oottayataa atayaaa agaatya oottayaa gaatyaay aatattigaa atattigaa tattagat taagyatyi gatyyaaa 6300 aayiyatta gaagaagaa agaataa atataag titagati agyayaya agaacag gatyatig gagaagaa atataga taagyata cayaataa ataatatta 6300 atayiyatta gaagaagaa gagataa ayayaya atayaacag 6300 atayiyatta gaagaagaa gagataa ayayaya atayaacag 6300 atayayayat gaagatag ataagaata cayaagaa ayaagaa ayaacag 6300 atayaataa ataaga taagaata cayaagaa ayaagaa ayaacag 6300 ataataataag ataagaa taagaata cayaagaa ayaagaa ayaacag 6300 ataataataag ataagaa taagaata cayaagaa ayaagaa ayaacag 6300 ataataataag agaataag ataagaata cayaagaa ayaacag 6300 ataataataag agaataag ataagaata cayaagaa ayaacag 6300 ataataataag agaataag ataagaata cayaagaa ayaacag 6300 ataataataag agaataag ataagaata cayaagaa ayaacaga 6300 ataataataag agaataga agaataa ayaagayaa ayaagaa ayaacag 6300 ataataataag agaataa agaataa agaagaaga gaaagaa gaagaagaa ayaa 6300 aacaagaaga aacaagaa aagaataa agaagaaga gaaagaaga gaaagaaga ayaa 6300 agaataata aacaagaa agaataa agaagaaga gaaagaa gaagaagaa ayaagaa 6300 agaataata aacaagaa aacaagaa aacaagaa ayaagaa ayaagaa ayaagaa 6300 agaataata aacaagaa aacaagaa aacaagaa aagaagaa agaagaa ayaagaa 6300 agaataata aacaagaa aacaagaa aacaagaa aagaagaa ayaagaa ayaagaa ayaa 6300 agaataata aacaagaa aacaagaa aacaacaa aacaagaa aagaagaa ayaagaa ayaa 6300 agaataata aacaagaa aacaagaa aaacaacaa aacaagaa ayaagaa ayaa 6300 agaataaa aacaagaa aacaag	gagtcagtga gcgaggaagc	ggaagagcgc ccaatacgca	aaccgcctct ccccg	cgcgt 5760	
gegestatt caaccocce tytyty cagytyce gegestatt ceaccocce gygygyce ggestatt caaccocce tytyty caagytyce gygygyde caagytyce gygygyde caaccocce tytytyce caaccocce ca	tggccgattc attaatgcag	ctggcacgac tagagtcccg	ctgaggcggc gtagc	aggtc 5820	
Andrew Server a sequence of the server of	agcegeecca geggtggtea	ccaaccgggg tggaacggcg	ccggtatcgg gtgtg	teegt 5880	
Ataytaytay ayattina gytenaat oogaagaac ttettytat ootacayta ootacaaata ytaaytyog ayataataa oogaagaac ttettytat ootacaytat tataytaytay ayattina gytenaat oogaagaac tattytaa ootaaa gyteygyaa ttattyog attettyo oygtataa oogaattaa taytogaa oogaattaa gyteygyaa ttattyog attettyo oygtataa ootaytataa taytogaaa oogaatta tattytaa attetugog ootaatta ootgataag ttoogaac oogaattaa gyteygyaa tattugog oytatata ootgataag ttoogaac ootgataaa gyteygyaa ootaya attetugo ootgataa ootgataag ootgatata gyteygyaa ootaya attetugo ootgata ootgataag ootgatataa gaagagyaa ootaya attetugo ootgata ootgataa ootgataa ootgataa gaagagyaa ootaya attetugoo ootgata ootgataa ootgataa ootgataa gaagagyaa ootagaata ootgataa gaagagyaa ootgataa ataactete gaagagyaa ayattaa ootgagaa gootoaa atayaa ootgataa tacctee gaagagyaa ayattaa taggaata gaagagyaa tayogyaga atagaacog gytegoaa ayattaa taggaata ootagagaa ootgataa tacctee ttegoataa ootgataa ootgagaa ootagaaa atactee ttegoataa tacctee tygtaataa ootgagaa ootagaaa atactee ooogagaag gootaac ootagagaa ootgagaa ayaogyaa tayooggac oocgegaag gootaaco tagataa ootgagaag oo gootaaa taccee ooogagaa gootaaa ootagagaa ootagaaa tacee gaagagaa ootagaa taccee gaagaagee tagaagaagee ootagaaa acacagaa gootaaco tagataa ootgagaag oo gootaaa tacee ooogagaa ayaagaa ootagaaa tagaagga tagaagaagaa tagaaggaa gaagaaa ootaacee gaagaagee aagagaagee tagaagaagee ootagaa gaagaaa acacagaa aagaagaa aagaagaa tagaaggaa googaagaagaa gaagaaaa acacagaa aagaata gaagaagaa tagaaggaa cagaagaaga aagaagaa acacagaa aagaata gaagaagaa dagaagaagaagaagaagaagaagaagaagaagaagaaga	ggcgctcatt ccaacctccg	tgtgtttgtg caggtttcgc	gtgttgcagt ccctc	gcacc 5940	
Artorization of the set of the	ggcacccgca gcgaggggct	cacgggtgcc ggtgggtcga	ctagtttatt aatga	tgatg 6000	
ttatgigaig actiggera gittagegi garciatog tattogora contrataag 6180 gggggggai tittageag atattitit ooggatato esgtottta togooga contrataag 6180 tagggaa garciagg ogtoosta ootsigato esgtottta togoogato 6300 taggaggao tosttag goggoosato ootsigato esgatget gatggaaa 6360 glagitacag gootootga attoiggat oogaaggao ogtatott ootsigagaa ootsigaaa 6400 esgagggao gaagataago ootsigaaga oostagaa ootsigaaga ootsigaaa ootsigaaga guiggeaca agottigato tigootoot aastgago ootsigaaga oostagaagaa ataacotoo 6600 guiggeaaa agottigato tagootoo goatocaaa gaigaagaa atagaacgi 6720 tatacottoo toggoota ootsigaaga egaggaa agoaggaa agoaggaa atagaacgi 6720 tatacottoo toggoota oostagaga agoaggaa tigagaagaa tigagaacgi 6720 tatacottoo tiggootaa coostaga agoaggaa tigagagaa agoaggaa agoaggaa agoagga 6860 staaatoog gootaata coosgagga agoaggaa tigagagaa tigagaacgi 6720 coosgegaag goottaga tooosgagga tagoogga tigacagaa igacagggoo 6960 gigeocagee gootacaga tigadgaa googgace gacagatag googgagaa 7020 coosoosga gootacaga tagoogaa googoogaa agooggaga googgagaa 7200 aacaagaacg doacoogaa agottoga tigoogace oiggoogga googgagaa 7200 aacaagaag agotoogga googtoog tactogoo cagootoo cigogoog atactogao 7320 aacaagaag agotoogga gaacoogo tactogoog coosoos cacacace 7320 aacaacgaag agotoogga agotocag toogoog coosoos cacacace 7320 aacaacgaag agotoogga aacactotig accaaggi googacga tactogaca 7320 aacaacgaag agotoogga agaactoca coosooso coosoo coosoo 7320 aacaacagaa agotoogga aacactotig accaaggi googacag cagaggigg 7440 tigggaggig tittigggg gittiggoog gatacciga taggodag googagag 7560 gaaggigtac agaagaato gaeggaca accactos accagag googaca cacacagag 7560 gaaggigac agaactoo goggooga aacactog accactaga figoogaca figoogaca 7560 gaaggigac agaaatoo gaaggaga accacco aacgaggiga googagag 7620 gittiggag gittigggag gittiggaga accactag accaccaa accacaa googaca 7560 agagaga gittiggag gittiggoog gaacciga tagooga figoogaca 7560 agagaga gittiggag gittiggaga accaccaa aacgagaga agoagaga 7760 cocactaga gaaata coosogaga aacaccoa aacgagaga agoagaga 7760 cocaccacga gaaca	atgatgatgc aggtgtttca	ggatgaaatc cgaaagcaac	ttgttgtatc cttca	cgatc 6060	
<pre>griggsgst thattiggs qittattig o grigstatto captactita togigcoggi figgsgst thattiggs qittattat origitatta origitatta figgsoggi figgsgst taattiggs qittattat origitattag tittogoriti cogogitatto figgsgsgst figgsogaa altoriggst cicgitaaga cagatgaa cigatgitaaa figgstigat gittattig orggogacaa ciccigatagi cagatgaa cigatgitaaa figgsogaa gococola altoriggst cicgitaaga cagatgaa cigatgitaaa figgsogaa gococola altoriggst gatgagacot thoogaa altoritic fig gargooda agottgat tagggooto tgococola altoritica accoratagi caatgigaa figgsogaa agottgat tagggooto tgococola altoritica accoratagi caatgigaa figgsogaa agottgat tagggooto tgococola altoritica accoratagi caatgigaa figgsogaa agottgat tagggooto tgococola datgatga cogogaca figocoggoo fittagootaa agottgat tagggooto tgococola tagaogaa tgocoggoo fittacocoto tiggtotat coggatotao gratocaao tgatogigaa tgocoggoo fittacocoto tiggtotat coggatotao cigatogaa toggogaa tgocoggoo fittacocoto tiggtotat coragagaa alegaggat toggagaa tgocoggoo fittaacattoo tiggtotat coragagaa alegaggoo tgatogaga tgocoggoo fittagooga gocacogoo gocacoo gocacoo figotocoto gococoo fi fittacocoto tiggtocaco gagaagoto cagogoo gocaggoo gocagaa fittagooga tagatacoo gagaagoto cagogoo gocaggoo gocacoo fi fittagooga gocacoo gagaagoto cagogoo gocaggoo gocacoo fi fittagooga gocacoo gogoo gocacoo fi gatootaa gocoggoo gocacoo fi fittagooagoo gocacoo gagaagoto cagogoo gocagad fi fittagooagoo gocacoo gocacoo fi gagoogoo gocacoo gocacoo fi fittagooga gatocoo gocacoo fi fittagooga gocacoo gocacoo fi fittagooga gocoo gocacoo fi fittagoogag gocacoo gocacoo fi fittagoogag gittagoo gocacoo fi fittagoogag gittagoo gocacoo fi fittagoogag gittagoo gocacoo fi figggoogag gittiggoo fi figggoogag gittiggoo fi figggoogag gittiggoo fi figggoogag gittiggoo fi figggoogag gittiggoo fi figggoogag gittiggoo fi figgoogaga gittiggoo fi figggoogag gittiggoo fi figggoogag gittiggoo fi figggoogag gocactag fi fi fi fi fi fi fi fi fi fi fi fi fi f</pre>	ctcccacatc gtgaggtgcg	agcaatccct gaagacgtga	agttccgaac cagct	atttt 6120	
tatggigaar teatteggig egteatte eregigaarg etgeagtete engeggaarg egteggaarg egteggaarg egteggegge egteggete engeggggeg egteggaarg egteggege egteggege egteggeg egtegge egteggeg egteggaarg engeggggg etgeggegge engeggggg etgeggggg etgeggggg etgeggggg egtegggggggg	ttcatgtatg actctggcca	cgtttggcgt gacctcatcg	tattcgccca ccgtt	ataag 6180	
tayigatti gagtactegg geggecaate etergatga ageagtege gatgygaaaa 6360 gagtggate gaagataag etergagate geaatgag etgteater etergatgat etgteater etteracegt 6480 cagagggaet gaagataag eterggate geaatgag etgteater etteracegt 6480 gatgegaae agetttgat tagggeet tergatgae eccateaaa atacettet 6600 getgeegaae agetttgat tagggeet tgeeterace atatgatga eccateaaa atacettet 6600 getgeegaae agetttgat tagggeet tgeeterace acategatga etggeggae tgeegggee 6720 tateeette tiggteata eccggaete geateeaaa tegtogggae tgeeeggee 6780 eccegtgeatg getataget tigettee etergagge tigeacage tgeatgae 6800 ataaatteeg ttaeettig egagetee geateeae tgategggee tgeetegge 6860 getgeeaeeg geteaeeg geagetee gegeteee gaeeggee geeggeegg eegetega 7020 eccegtgeatg getataget tigettee tageetee tggeeggge egageteege 7020 eccegtgeatg getaceeg geagetee gegeteee eageggee geeggaegtee 7020 eccegteeag geteaege geggeege gegeteee ageeggeeg geegetegat 7140 eccegeteega atgtaeee gaagaaee eageggee teggeegge geegeaga 7200 agagtgaaea accacegae accecter etggeege geteeae eacedee 7380 geageaagee eaceerege eegegeege teggeege geteegae faceerege 7320 accacegeae accecter ag eegegeeg eegegeeg geteegaa eaceerege 7320 agaggaagag getteggeg gataeereg geeggeeg geteegaa eaceerege 7320 agaggaagag getteggeg gataeereg geeggeega teggeega faceerege 7320 agaggaagag getteggeg gataeereg geeggeega geeggeag faceerege 7320 agaggaagag getteggeg gataeereg geeggeag ateggeag faceerege 7320 agaggaagag getteggeg gataeereg geeggeag ateggeag faceerege 7320 agaggaagag getteggeg gataeereg geeggeag ateggeag faceereg 7320 agaggaagag getteggeg gataeereg geeggeag ateggeag faceereg 7320 agaggaagag getteggeg gataeereg geeggeag ateggeag faceereg 7320 agaggaagag getteggeg gateereg geeggeag acceareereg 7320 agagegaag getteggeg gateerege geeggeag ateggeag faceereg 7680 agtgeeregee dagaaate gaaegatee geeggeag ateggeag faceereg 7680 agtgeeregee geegettge acceareg geeggeag ateggeag faceerega 7680 agtegeegae geesttge ateggeag accearee aegegeege geegategg 7620 gateerege geegaetgg ateggeag accearee aegegee	ggtggggatc tttattgcag	atattttgtc cgtgatatcc	cagtccttta tcgtg	ccggt 6240	
<pre>gtagttacacg gcotoctgat attcsgagat ctcgtagaga ccgtatagaac cgtattttt 6420 aaatggcatc ctgtactttg ccggagacte gtcatagag ctgttacatt ccttacaccg 6480 ccagagggat gaagatago ctcoggata gtcatagag ctgttacatt ccttacaccg 6600 gttgcagaga agcttgat ttagggoct tgoctacaca tagtagga tagtagaga 6600 tttagactga tcoggtact cgggatca gacgacaca tgatcgtaga atgacacgg 6720 tatccctc ttggtcatt cccggagaga agcgggt tgocagggg tgocagacg gtgtcoggg 6780 cccggtgatg gtcattagc ttggttttc ctcagagggt tgocagcog gacgttcog 6780 cccggtgatg gtcattagc ttggttttc ctcagagggt tgocagcog gacgttcog 6780 cccggtgatg gtcattagc ttggttttt tatgattc tgatcatg ccgtcoggg 660 gtgcacagce gtcacaget gcgtcocaca tagagggt tgocagcog gacgttcog 6960 gtgcacagce gtcacaget gcgtcocaca tagaggt gtcaagcog gacgttcog 6960 gtgcacagce gtgagcgac ggtgcoge ggtgcoget ggtcocaca gccggggg cgtgggg 7020 ccccctga atgtacacc gaagagtca cagegtcoc ctgggccg gatcgatcg 7080 accacggacg gtgagcog cggggctca tacggcgg gtccgatcg gccgggg atactgac 7080 accacggaga agctcog ggggctca tacggtcg cgcggcg taccaccc 7720 agggggat aggacaac accctg cctggcgcg gtcgattg gcggggag 7220 aggaggag gttcoggg gataccgcg taccaccc 7720 agggggat gagtaccg accccttg caccaggg gcgcggg gcgtggag 7220 aggaggag ggtcocgg aggacac tacggaga taggcggg gcggggg gcggggag 7220 aggaggag ggtcocgg aggacacc agggtcog tggtcgag gcggggg gcggggag 7220 aggaggag ggtcocgg gagaccgc gcggggcg gtcgattg gcggggg gcggggag 7220 aggaggag ggtcocgg gagaccg gcggggg gtcgagag gtcggaga 7200 aggaggaga ggtcocgg gacaccctg ccggcgcg gcgcggg gcggggg 7220 acccagga gagaaac gaaggaca gcggggg ggtgggg gcggggg gcggggg gcggggg 7220 acccagga gagaaac gaaggaca gcggggg ggtgggg gcgggga gtcgagg 7220 acccagga gagaaac gaaggaca tgocgga gtcgagag 7200 aggaggag gtttggg gg gcgggg gaccctg gcgcgcg gcacgagg gcgggg 7220 acccagga gagaaac gaaggaca gcggcgg gcgacgg gcgggg 7220 acccagga gagaaac gaaggaca gcggggg gtgggg gacggg gacgggg 7220 acccagga gagaaac gaaggaca gcggggg gtgggg gacgggg gacgggg gacgggg gacgaggg gacgaggg gacgggg gacgagag 7200 aggaggag gtttggg gg gcgggg gacggg gacggg gacgggg gacggag gacgaggg gacgagag gacgggg gacgagag ga</pre>	tatggtgaac tcattcgggc	cgttcattat cctgtatacg	tttcgccttt ccgcg	tattc 6300	
aatggaata citti caggagaata gaataaga citgtaatagaga cigtacata cattaagaa citggaata gaagaagaa gaagagaga gaagaagaa aagaaggaa taagagaga citagaagaa aagaaggaa tagaaggaa tagaagaga tagagagaa tagaaggaga tagaagaga tagagagga tagaagaga tagaaggag tagaagaaga gagaagaa tagaaggag gaataagaa aagaaggaa tagaagagg aagaagaga tagaagagg tagaagaaga gagaagaga tagaagagg gaataaga gagaggaa tagaagag gagataaga gagaagaga tagaagag gaataaga gagaggaa tagaagag gagaacaa aacaatag aacaaga gagaggaa tagaagaga aagaagga tagaagag gataaaga gagaaga gagaagga gataaga gagaaga gagaagga gataagaga gagaagga tagaagga gagaatag gagaacaa aagagga gagaagga tagaggag agaacaa aagagga gagaaga aagaagga tagagaag gagaaga aagaagga tagagaag gagaacaa aagagga gagaata agaagga gagaacaa aagagga gagaagga aagaagga tagagaag gagaacaa aagaagga gagaagga aagaagga aagaagga gagaagga gagaagga gagaagga gagaacaa aagagga gagaacaa aagaagga aagaagga gagaacaa aagaagaa aagaagaa aagaagaa aagaagaa aagaag	tagtgatttg agtacctcgg	gcggccaatc ctctgatctc	agcagatgct gatgg	taaaa 6360	
cagaggact gaagtaag oftooggata gatgagoot ttoagatgat ootggtaott 6540 gactgaggact gootgooca gootocac atatgatga ocototacaaa atacottoo 6600 gttgoogaa agottgat ttagggoot tgootocac actgatgag atagaacgt 6600 tttagactg tooggtoo ggatocac goatocaac tgatogga atagaacgt 6720 tatocottoo ttggtoata ocotgagag aagoaggaa togtgggac tgoocggoo 6780 ocoggtogg gtoatagot tgottot ocotagggg ttgocago tgocacgo 6780 ocotgago gtocacgoo gotgagga agoaggga tgotgagag tgocagoog 6780 ocotgagoog gtocacgoo gotggoot tgottot otgotggg ttgocagoo gootgoot 6900 ottotagacgo gtocacgoo gotgootac gadotgag gtocagoog googgago 7020 ocogeotoga atgaacace gagaagote cagotoca googgoog gotgggage 7020 ocogeotoga atgaacace gagaagote cagotoca googgoog gatacega 7020 ocogeotoga atgaacace gagaagote cagotoca googgoog gotgggaga 7200 aacacgoog cacacegaa taacgatteg googgoot gattegg googgacga 7200 agaggagaa accacegae taacgatteg googgoog gtocgate gatecgaca 7080 agagaggae agotecogg gatacege ttgegoog gtocgate gatecgae 720 aggoaggae caaceegaa accetteg tedegoeg gacegae taccegae 720 aggoaggae caaceegaa accetteg tedegoeg gtocgate tacceceg 720 aggoaggae agotecogg gatacege tedegoog gtocgae taccece 720 aggoaggae agotecogg gatacege tedegoog gtocgae taccece 720 aggoaggae agotecogg gatacege tedegoeg gtocgae taccece 720 aggoaggae agotecogg gatacege tedegoeg gtocgae taccece 720 aggoaggae agotecogg gatacege tedegoog gtocgae taccece 720 aggoaggae agotecogg gatacege tedegoog gtocgae gaggetg 740 tgggaagag gttttgggg gtugoogg gatacega taggoaga ftocaaaag caggggtg 740 tgggaagag gttttgggg gtytgoog gatacega taggae gtocgae ftocgae 750 agattaatt tocatatag ctoggoege tegatoga taggae ftoggaag fgoggagae 760 gattacece tgaaacega accecteg gacegae accecta aacgaggae ftoggae 760 agttytee cgoacego gttoegaa aacaete aacgagta gtoggaa ftocgae 760 agttytee cgoacego gttoegaa aacaete aacgagea ftoggae ftoggae 760 agotegae agotecoga atgaegae googae gacedae gacegae 770 agotataatt tocataag caegegae gtoggae googae atcegae gtoggaegae 770 agotataat tocatag caecegae gacegae googae googae gaceeae 7800 agocaecegae gaceateg aacaetege g	gtagttcacg gcctcctgat	attctggatt ctcgtaagat	ccagatgaac cgtat	tttt 6420	
gactgraft jocagragora gogotocar i tatgatgar o coatoaaa atacottoto 6600 gitgoogaa agottigat itagggoot igoototoc acacotagi caatigigaa 6660 tittagactga looggitoot oggatotae goatoaaa togitggaga tagaacegi 6720 tatocotto itggoata cootgagaga aagoaggaa logigggaa igoocggoo 6780 cootgagoat gitatagot itgottot o coagggot itgoacagot igaataaat 6840 ataaattee ittacettig egitgette tatgoattee igoocggoe googgage 6960 gitgooagee gitgaegee geigteege geigteege geocggoge eegitgege 7020 cooggoogga gitaacee gagaagete caegeeee eigeeegigee atacegae 7080 aecaegee gitgaegee geigteege geoggeee eigeeegee geoggeee itageeegig eigeeegige 7140 agagaigaa acaecegae taacgatte eigeeegige geocgae aceeceee 7260 agagaigaa acaecegae aceectegi eigeeegige getegategi eigeegigee 7320 aagaegagee eigeteegig gitgeeegig gataceee eigeeegigee itaceeee 7260 agaegagae acaecegae aceectegi eigeegigee gitgeeegi eigeeigaegi 7200 agaegagae aceecegae aceectegi eigeegigee gitgeegigee isaetegae 7200 agaegagae ageteegig agaegeee eigeteegit eigeeegigee googgeegi 7200 agaegagae aceecegae aceectegi eigeegigee gitgeegigee aceecegie 7260 agaegagae ageteegig gitgeeegi gaaceee eigeegigeig eigeegigeig 7320 agaegaagee ageteegig agaegee goeggeee eigeegigeig eigeegigeig 7320 agaegaagee caaceegae aceectegi eigeegigei eigeeligeei eigeegigeigei 7320 agaegaage gitteigigei gitgeeegig giteeaaaag eagggitig 7440 tigggagagi gittiggegi gitgeeegig eigeegige atagee aggegigei 7560 agataatt toeataag eigegeegi eigeegiae atageatig geoggaegi 7560 agataatt toeataag eigegeegi eigeegiae atagegaa giteeegigi 7560 agaeceaegi eigeategi eigeegigei gitgeaegi aceecee aacegaegi gitgeegigi 7460 tittagei tigaedigi aaceece aacegaegi eigeegiae gitgeegigi 7740 tittategia tigaetegi taegitga geogegige gitgeegig gitgeegigi 7740 tittategia tigaetigi tigaegi aceecee geogeegie eigeegigei aceecee 7860 ageecaecegi eigeegie gitgeegee gitgeegee geegie geegie aceecee 7860 ageecaecegi eigeegie gitgeegee gitgeegee geegie geegie aceecee aceeceecee 7860	aatggcatct ctgtactttg	ccgggagctc gtcaatgagc	ctgttcatct ccttc	accgt 6480	
gitgorgaacagotttgatettagggoetetgoctotecacaccatagtcatattgtgaacatattgtgaagitgorgaacagotttgatetgggtoetecggatetacegeategggaatggtogggaacataggageecatattgtgaatatecetteettggteataceetggagaaaageaggtaategtgggaatggeeggeecataataatcataataatceeggtgeatgiteatagetttgetteettgeatagetgeaggeecataataatcataataatcataataatecataataatecataataatecataataatecataataatecataataatecataataatecataataatecataataatecataataatecataataatecataataatecataataatecataataatecataate <td>cagagggact gaagataagc</td> <td>ctccggatac gatgagccct</td> <td>ttcagatgat cctgg</td> <td>tactt 6540</td> <td></td>	cagagggact gaagataagc	ctccggatac gatgagccct	ttcagatgat cctgg	tactt 6540	
ttagactga tccggttcc cggatctae geatceaa tgatcgtag atagaaceg 6720 tatccotto ttggtcata cccggaga ageaggta tcggggaca tgccggggc 6780 cccgtgcag gtcattage ttgettte tatgettee tgatcaage tgeaageeg gegedee ttaccott gegeg gteaagee gegtgeeg ggeteete ggeteete ggecggg egggggg 6960 cccgetega atgtacaee gagaagee egggeeg ggeteete tggacggg egggggg 200 ccgeetega atgtacaee gagaagee egggeege getegete geetege geetege atactegae 7020 ccgeetega atgtacaee gagaagee eggegeeg getegete geetege geetege atactegae 7020 ccgeegege tegattege eggegeteg tegetgeeg getegete gateege geetege atactegae 7080 aacaeegeae acceegea taacgateg geeggeete datteggeeg geetegat geeggeeg atactegae 7200 agagtgaaca accaeegea caceette teggeege getegete getegege geetegate geeggeege tegetege 7200 ageegagge ageeteegg agtaceeg tegegeeg tegegege geegegee tegetege tegetege	gactgcgtat gccagcgcca	gcgctccacc atatgatgac	cccatcaaaa atacc	ttctc 6600	
tatcoctto tiggtcata coctgagaga aagcaggta togtgggaca tgocogggc 6780 cocgigcatg gtcattaget tigettee etcagggget tigeacaget gtaataaat 6840 ataaateeg titacettig egtagttee taggatee tgaeetggg egedeecet 6900 etetgaegee gtocaegetg eetecteag tgaegtgagg tgeaageeeg gaegtteege 6960 gtgeeaegee gtgageege gegtgeegte ggeteeete etgggeegg eegtgggage 7020 eegeetegat atgtaeaee gagaagetee eagegteete etgggeegg ataetegaee 7080 accaegeeage eacaeegeae taaegatteg geeggeege gateeggeeg gaeteegae 7020 aggetggage tegattegge eggegetega teeggeege getegateg geegageag 7200 aggetggaea accaeegae taaegatteg eeggegee gtaeegeege geegageag 7200 aggetggaea accaeegae aegetteege tetggeege gtaeegaee taeeteege 7260 aggetggaag ageteecgg ataeteage eggeggeeg ataeteagae 7320 aaggetgaaea accaeegae aegetteege tetggeege gtaeegaee taeeteege 7320 aaggetgaage ageteeegg acaeedege geeggege gteegaeg gettegate 7380 geagggttee agaagaaate gaaegatee geeggeaeg gtteaaaaag eagggttg 7440 tggggaggg gtttigggg gtgtegeegg gteetgaa tagtgatg ggegggege 7560 agttgeeege atgaaateeg aaeggggge gteetgage atagteagae gteeggaeg 7560 agttgeeeg agaagaate teeggeege gaeegae atagteegae ateggeegg 7620 gtgttgette egeaeegge gteeggeeg ateggaeat gaeeggeatg 7560 agttgeeega ageettgge ateggeeg ateggaeat gaeegaeatg geeggateg 7620 gtgttgette egeaeegge gteegaeg accaeete aaegaegge 7740 tetategtea tegaetgee ataeegttage geeggeeg egeegae gteegaeg 7740 tetategtea tegaetgee ateegttage geeggeeg geegeae accaee 7800 gaeeateegg egeegaeatg gteegaea tegeegteeg geegeae acaeeatee 7800	gttgccgaac agctttgatc	ttagggcctc tgcctcttcc	acaccatagt caatt	gtgaa 6660	
cccggtgcatg gtcattagct tigcttictc ctcagggggt tigcacagct tgtaataaat 6840 ataaatteg titaecttig egtagttite tatgeattee tgatecatgg eegeteede 6960 gtgeeaegee gteeaegetg eeteeteag ggetgeege ggeteetea geeegggegg eegtggggae 7020 cegeetegat atgtacaece gagaagetee eageggegete gatteggeeg atactegaee 7080 accaegeeage cacaecegea taacgateg geeggeete gatteggeeg geetgatteg 7140 eggeeggegg tegategge eggegetega tteggeegge getegatteg geeggeaga 7200 aggatgaaca accaecegae acgetteege tetggeegge gateegateg geeggaega 7200 aggetgaage ageteeegg agtaeegee tetggeegge gateegateg geeggaega 7200 aggetgaage ageteeegg agtaeegee tetggeegge gteegatteg geeggeega 7320 aegeeaage eageteegg agtaeegee tetgeegge geeggeteg atteegeeg 7320 aegeaaage eaceeegga agtaeegeg taeteegee eegeegge eregategateg 7320 aegeaaage eaceeegga agtaeegeg geeggeaag gteeaaaag eaggggtgg 7440 tggggaggag gtttggggg gtgtegeegg eggetegaa tagtgatg ggeeggegea 7560 agttgeeege agaateege aaegggege eggaetega tagtgaegg geeggatgeg 7620 gtgttgette egeaeegge gteeggeeg geegaeteg ateggeagg geeggatgeg 7620 gtgttgette egeaeegge gteeggeeg acaeaeete aceggaeg ateggeagg 7620 gtgttgette egeaeegge gteeggeeg aeaaeeetee aeegaggte geegaetgg 7620 gtgttgette egeaeegge gteeggeeg aeaaeeetee aaegaggteg gteeggaeg 7620 gtgttgette egeaeegge ateggeegg aaeaaeeetee aaegaggteg gtaeeggatg 7680 ageeegaeg aegeattgge ateggeeg geegateg geegattega geegaatge 7680 ageeeggaeg aeeattgge ategegtae geeggatge geegattega geaaeeatee 7800 gaeeateegg eegeaattg ggttgeedaa tegeegtee geegaetega eategeetee 7800	tttagactga tccggttcct	cggatctacc gcatccaaac	tgatcgtaga ataga	accgt 6720	
ataaattoog titaacettig ogtagttite tatgoattoe tgatocatgg oogtocotti 6900 ototgaogoe gtocacegetg ootoctoaeg tgaegtgagg tgoaagecoog gaegttoogo 6960 gtgocacege gtgagecege gegtgeegte ggeteectea geoegggegg oogtggage 7020 cegeetegat atgtaeacee gagaagetee cagegteete etgggeegg atactegaee 7080 acceaegeaeg cacaecegeae taaegatteeg geoggegete gatteggeeg gegetegatt 7140 ceggeeggeeg tegattegge eggegetega tteggeegge getegatteg geoggaega 7200 agagtgaaca accaeegaee acgetteege tetgegeeg gtaecegaee taeeteeceg 7260 ageetegaag ageteecegg agtaecegee gtaecegaee taeeteeceg 7320 aegeaaagee caaeceegae acgetteege tetgeegee gtaecegaee taeeteeceg 7320 aegeaaagee caaeceegae acgetteeg tetgeegee gtaecegaee taeeteeceg 7380 geagggttee agaagaaate gaaegateea geegegeaag gteeaaaag caggggttg 7440 tggggaggg gttttggggg gtgtegeegg eggeetegat atggeetggt 7500 cegaataatt teeatatage eteggeegg agaeetega atagtegat ggeegatgg 7560 agttgeeee agaagaaee gaeeggeeg eggeetega atagtegae geegaeat ggeegatgg 7620 gtgttgette egeeegge gtteegeae gaeegaetee acegeege ateggeaat ggeegatgg 7620 gtgttgette egeeegge gtteegeae gteggeega acaeetee aaegaggte gteecegae 7740 tetategtea tegaetgea teaegttgae geegeatge geegeateg geaeceae 7800 gaeeateege eceaaecaegt ggetgeeee gaeegeeg geegeatega eateeggatg 7860 tetategtea tegaetgega teaegttgae geegeatee geegeateg geegaetge 7860 gaeeateege eceaaecaegt ggetgeeee gaeegeeg geegeatee ateeggatgg 7860 tetategtea tegaetgega teaegttgae geegeatge geegeatee ateeggatgg 7860	tatcccttcc ttggtcatat	ccctgagaga aagcaggtaa	tcgtgggaca tgccc	gggcc 6780	
cictgaegee giceaegetg ecteeteaeg igaegigagg igeaageeeg gaegiteege 6960 gigeeaegee gigageegee gegigeegie geeteetea geeeggeegg eegiggage 7020 cegeetegat atgitaeaeee gagaagetee eagegieete gatteggeeg gegetegat 7140 eeggeeggee tegattegge eggegetega tieggeegge getegateg geegaegaa 7200 ageategaage aceaeegae aaegettege teiggeegge giteegateg geegaegaa 7200 ageagagigaaea aceaeegga agtaeegeeg tetiggeegg getegateg geegaegaa 7200 ageategaage ageteeegg agtaeegeeg tetiggeegg egteegateg 7260 ageaggagaae eaaeeeggag agtaeegeeg tetiggeegg etteegaee aceateeege 7320 aegeaagee ageteeegg agtaeegeeg taeteaeeeg eeggeggeteg etteegeeg 7320 aegeaggittee agaagaate gaaegateea geeggegaag giteaaaaag eaggggitgg 7440 tiggggaggag gittigggg gigtegeegg gataeetgaa tagtegitg tittigegitag 7500 eggaataatti teeataage eteggegeg eggeetgaat atggeegaatg geeggatege 7620 gigtigteete egaaetege aaeggggge gigeigageg ateggeaatg geeggateg 7620 gigtigteete egaaetege aaeggggge gigeigage ateggeeatg gigeeggateg 7620 gigtigteete egaaetege aaeggggge gigeigage ateggeaatg geeggateg 7620 gigtigteete egaaetege aaeggggge gigeigage ateggeeate geeggateg 7740 tetateegea tegaateega teeegitige geegeateg geegeateeg 7740 tetateegea tegaetegea teeegitige geegegateg geegeatega geaeeete 7800 gaeeeateegg egeegaaetg gittegeaea tegeegteeg geegeatega 7800 gaeeateegg egeegaaetg getigeegae aeegegeegeegeegeegeegeegeegeegeegeegeege	cccgtgcatg gtcattagct	ttgctttctc ctcaggggct	ttgcacagct tgtaa	taaat 6840	
gigocaagac gigagcegee gegtgeegte ggeteectea geeegggegg eegtgggage 7020 cegeetegat atgtacaece gagaagetee eagegteete etgggeegge ataetegaee 7080 accaegeaeg eacaeeggee taaegatteg geeggeete gatteggeegg geetegatt 7140 eggeeggege tegattegge eggegetega tteggeegge getegatteg geegageaga 7200 ageatggaaea accaeeggee acgetteege totgegegee gtaecegae taeeteeege 7260 ageetegaage ageteeeggg agtaeegeeg taeteaeceg eetggetea ecaeteeege 7320 aegeagagtee eaaceegge acaeetteg eetggeegge etggetega taeteege 7320 aegeagagtee agaagaaete gaaegatee geeggeeg getegateg geeggegge 7320 aegeagggttee agaagaaete gaaegateea geeggegeag gtteaaaaag eaggggttgg 7440 tggggaggag gttttggggg ggtegeegg gataeetgaa tagtegatg ggeeggateg 7500 eggaataattt teeatatage eteggeegg eggeetegaa tagtegatg ggeeggateg 7560 agttgeeee aaegegege gteegegee gaeaeetee aeegggte gteeeggat 7620 fgggttgette egeaeegge gteegegeg aaeaaeetee aaegaggte gteeggatg 7680 ageeegegae aegeattege atgeeggea geegeattega geaeedge 7740 tetateegtea tegaetgega teaegttga geeggatge geegeatega geaeetee 7800 gaeeeateegg egeegaaetg ggttgeaeaa tegeegteeg geegeatega eateeggatgg 7860 tetateegtea tegaetgega teaegttga geeggetee gaetgaeege acteeggeteg 7860 gaeeeateegg egeegaaetg ggttgeaeaa tegeegteeg geegeatega eateeggatg 7860 tetateegtee tegaeteegg eggeegee gaetgeee gaetgaeege acteeggeteg 7860	ataaattccg tttacctttg	cgtagttttc tatgcattcc	tgatccatgg ccgct	ccctt 6900	
cogoctogat atgtacacce gagaagetee cagogteete etggeegeg ataetegaee 7080 accacegeaeg eacacegeae taacgatteg geeggegete gatteggeegg geetegatte 7140 eggeeggege tegattegge eggegetega tteggeegge getegatteg geegageaga 7200 aggagtgaaea accacegaee acgetteege tetgegeege gtacegaee taeeteeceg 7260 ageetegaage ageteecegg agtaeegeeg taeteeaeeg eetggetee ceateeaeeg 7320 aegeaaggee caaceegage acacetettg eaceagggg eetggetee ceateeaeeg 7320 aegeagagetee agaagaaate gaaegateea geegggeaag gtteaaaaag eaggggtgg 7440 tggggaggag gttttgggg gtgtegeeg gataeetgat atggetttgt tttgegtagt 7500 egaataattt teeatatage eteggegege eggaetega taggeaatg ggeggageg 7620 agetegeege acgeatege acaeetee aaegaggtea gtaeeggat g7620 gtgttgette egeaeegge gttegegae gaeeaeetee aaegaggtea gtaeeggat 7740 tetategtea tegaetgeg atgeegtae geegeatee aeegaegte 7740 tetategtea tegaetgeg teegetgae geegeateg geegeateeg 7740 tetategtea tegaetgeg tegegtee geegeateg geegeateeg 7740 gaeeateegg egeegaaetg ggttgeaeaa tegeegteeg geegeateeg 7740 tetategtea tegaetgeg tegegtae geegeateg geegeaee 7780 gaeeateegg egeegaaetg ggttgeaeaa tegeegteeg geegeaee 7780	ctctgacgcc gtccacgctg	cctcctcacg tgacgtgagg	tgcaageeeg gaegt	teege 6960	
accacgcacg cacaccgcac taacgatteg geeggegete gatteggeeg gegetegat 7140 cggeeggege tegattegge eggegetega tteggeegge getegatteg geegageaga 7200 agagtgaaca accacegace acgetteege tetgegegee gtaccegace taecteege 7260 agetegaage ageteeggg agtacegeeg taeteaceeg eetgegete coateeaceeg 7320 acgeaaagee caaceegage acacetetg eaceaggtg eegaeegtgg ettteegete 7380 geagggttee agaagaaate gaaegateea geegegeaag gtteaaaaag eaggggttgg 7440 tgggggaggag gttttggggg gtgtegeegg gatacetgat atggettgt tttgegtagt 7500 egaataattt teeatatage eteggegege ggeetegaat tagtegatgt ggeegggeae 7560 agetegeaege accettge aacagetega ateggeagg ateggeatg gteegaegg 7620 gtgttgette egaaeeggee gttegegeg gateacete aacegaggtea gtaceeggat 7680 ageegegaeg accettgge ateggeget gegegeatte eacegeeg gttgetegga 7740 tetategtea tegaetgeg teegetgae gteeggeate gegeatteeg geeaeectee 7800 gaeecateegg egeegaaetg ggttgeeee geegegate geegeeteeg 7860 ageecateegg egeegaaetg ggttgeeee geegegate 7860 ageecateegg egeegaaetg ggttgeeee geegegate 7860 tetategtea tegaeetgeg teegegeae tegeegeege 7860 ggeeeteegge egeegaaetg ggttgeeee 7860 ageecateegg egeegaaetg ggttgeeee 7860 gaeecateegg egeegaaetg ggttgeeee 7860 ggeeeteege egeegaaetg ggttgeeee 7860 gaeecateegg egeegaaetg ggttgeeee 7860 gaeecateegg egeegaaetg ggttgeeee gaeegeegee 7860 tegeteggee eeeaecgg egeegaee geegeegee acegegeegee 7860	gtgccacgcc gtgagccgcc	gcgtgccgtc ggctccctca	gcccgggcgg ccgtg	ggagc 7020	
cggccggcgc tcgattcggc cggcgctcga ttcggccggc gctcgattcg gccgagcaga 7200 agagtgaaca accaccgacc acgettccgc tctgcgcgcc gtacccgacc tacetccccgc 7260 agetcgaage ageteecggg agtacegeeg taeteaceg cetgtgetea ceateeaceg 7320 acgeaaagee caaeeegage acaeetettg caeeaaggtg cegaeegtgg ettteegete 7380 gcagggttee agaagaaate gaaegateea gegeggeaag gtteaaaaag caggggttgg 7440 tggggaggag gttttggggg gtgtegeegg gataeetgat atggettgt tttgegtagt 7500 egaataattt teeatatage eteggegge eggaetegaa tagttgatgt gggegggeae 7560 agttgeeea tgaaateega acaegtgg gtgetgageg ateggeaatg ggeggatgeg 7620 gtgttgette egeaeegge gttegegaeg acaeaeetee aaegaggte gtgeetgagg 7680 ageeeggaeg acgeattgge atggeggae gtegageat caeegaege gtgetegga 7740 teetategtea tegaetgega teaegttgae geegeatteg geegateega 7740 tetategtea tegaetgega teaegttgae geegeatge geegeatega 7740 tetategtea tegaetgega teaegttgae geegeatge geegeatega 7860 gaeeacaeegg egeegaaetg ggttgeegee gaeegaetega 7860 teetategge eceaaeegt gtgeegeae gaeegeege acaegagge 7860	ccgcctcgat atgtacaccc	gagaagctcc cagcgtcctc	ctgggccgcg atact	cgacc 7080	
agagtgaaca accaccgacc acgetteege tetgegegee gtaecegace taeeteege 7260 agetegaage ageteeggg agtaecgeeg taeteaceeg eetgtgetea eeateeceg 7320 aegeaaagee caaeeeggg agtaecgeeg taeteaceeg eetgtgetea eeateeceg 7380 geagggttee agaagaate gaaegateea gegeggeag gtteaaaaag eaggggttgg 7440 tggggaggag gttttggggg gtgtegeegg gataeetgat atggetttgt tttgegtagt 7500 egaataattt teeatatage eteggeeggt eggaetegaa tagttgatgt gggegggeae 7560 agttgeeea tgaaateege aaeggggge gtgetgageg ateggeaatg ggeggatgeg 7620 gtgttgette egeaeegge gttegegaeg accaeetee aaegaggtea gtaeeeggatg 7680 ageeeggaag acgeattgge aatgeggtae gtegageat eaeegaggtea gtaeeeggat 7740 tetategtea tegaetgega teaegttgae geegeateg geegettega geaaeeatee 7800 gaeeeteeggee eeaeeeg gtgeegeae geegeateeg geegetega 7860	accacgcacg cacaccgcac	taacgattcg gccggcgctc	gattcggccg gcgct	cgatt 7140	
agetegaage ageteeegg agtaecegeeg taeteeaeceg eetgegee eetgegegeegeegeegeegeegeegeegeegeegeegee	cggccggcgc tcgattcggc	cggcgctcga ttcggccggc	gctcgattcg gccga	gcaga 7200	
acgcaaagce caaceegage acacetettg caceaaggtg eegacegtgg ettteegete 7380 geagggttee agaagaaate gaacgateea gegeggeaag gtteaaaaag eaggggttgg 7440 tggggaggag gttttggggg gtgtegeegg gatacetgat atggetttgt tttgegtagt 7500 egaataattt teeatatage eteggegegt eggactegaa tagttgatgt gggegggeae 7560 agttgeeeea tgaaateege aacgggggge gtgetgageg ateggeaatg ggeggatgeg 7620 gtgttgette egeaeeggee gttegegaeg aacaacetee aacgaggtea gtaeeggatg 7680 ageegegaeg acgeattgge aatgeggtae gtegageat eaeegaege gegeattega geaeeaee 7800 tetategtea tegaeetgea teaegttgae geegeatge gegeattega geaaceatee 7800 gaeeateegg egeegaaetg ggttgeeaea tegeegeeg geegeaee ateggatgg 7860	agagtgaaca accaccgacc	acgetteege tetgegegee	gtacccgacc tacct	cccgc 7260	
gcagggttcc agaagaaatc gaacgatcca gcgcggcaag gttcaaaaag caggggttgg 7440 tgggggagga gttttggggg gtgtcgccgg gatacctgat atggctttgt tttgcgtagt 7500 cgaataattt tccatatagc ctcggcgcgt cggactcgaa tagttgatgt gggcgggcac 7560 agttgcccca tgaaatccgc aacggggggc gtgctgagcg atcggcaatg ggcggatgcg 7620 gtgttgcttc cgcaccggcc gttcgcgaga aacaacctcc aacgagggtca gtaccggatg 7680 agccgcgacg acgcattggc aatgcggtac gtcgagcatt caccgcacgc gttgctcgga 7740 tctatcgtca tcgactgcga tcacgttgac gccgcgatgc gcgcattcga gcaaccatcc 7800 gaccatccgg cgccgaactg ggttgcacaa tcgccgtccg gccgcgcaca catcggatg 7860	agctcgaagc agctcccggg	agtaccgccg tactcacccg	cctgtgctca ccatc	caccg 7320	
tggggaggag gttttggggg gtgtcgccgg gatacctgat atggctttgt tttgcgtagt 7500 cgaataattt tccatatage eteggegegt eggaetegaa tagttgatgt gggegggege 7560 agttgeeeea tgaaateege aaegggggge gtgetgageg ateggeaatg ggeggatgeg 7620 gtgttgette egeaeeggee gttegegaeg aaeaaeetee aaegaggtea gtaeeggatg 7680 ageegegaeg aegeattgge aatgeggtae gtegageatt eaeegeaege gttgetegga 7740 tetategtea tegaetgega teaegttgae geegegatge gegeattega geaeeetee 7800 gaeeateegg egeegaaetg ggttgeaeaa tegeegteeg geegeetae eateggatg 7860	acgcaaagcc caacccgagc	acacctcttg caccaaggtg	ccgaccgtgg ctttc	cgctc 7380	
cgaataattt tocatatago otoggoggg oggactogaa tagttgatgt gggogggcac 7560 agttgococa tgaaatoogo aacggggggo gtgotgagog atoggoaatg ggoggatgog 7620 gtgttgotto ogoacoggoo gttogogaacg aacaacotoo aacgaggtoa gtacoggatg 7680 agcogogacg acgoattggo aatgoggtac gtogagoatt cacogoacgo gttgotogga 7740 totatogtoa togactgoga toacgttgac googogatgo googoattoga goaacoatoo 7800 gaccatoogg ogoogaactg ggttgoacaa togoogtoog googogoaca catoggatgg 7860 tggotoggoo ocaacoacgt gtgocgcaco gacagogooo gactgacgoo actgogotac 7920	gcagggttcc agaagaaatc	gaacgatcca gcgcggcaag	gttcaaaaag caggg	gttgg 7440	
agttgcccca tgaaatccgc aacggggggc gtgctgagcg atcggcaatg ggcggatgcg 7620 gtgttgcttc cgcaccggcc gttcgcgacg aacaacctcc aacgaggtca gtaccggatg 7680 agccgcgacg acgcattggc aatgcggtac gtcgagcatt caccgcacgc gttgctcgga 7740 tctatcgtca tcgactgcga tcacgttgac gccgcgatgc gcgcattcga gcaaccatcc 7800 gaccatccgg cgccgaactg ggttgcacaa tcgccgtccg gccgcgcaca catcggatgg 7860 tggctcggcc ccaaccacgt gtgccgcacc gacagcgccc gactgacgcc actgcgctac 7920	tggggaggag gttttggggg	gtgtcgccgg gatacctgat	atggetttgt tttge	gtagt 7500	
gtgttgcttc cgcaccggcc gttcgcgacg aacaacctcc aacgaggtca gtaccggatg 7680 agccgcgacg acgcattggc aatgcggtac gtcgagcatt caccgcacgc gttgctcgga 7740 tctatcgtca tcgactgcga tcacgttgac gccgcgatgc gccgcattcga gcaaccatcc 7800 gaccatccgg cgccgaactg ggttgcacaa tcgccgtccg gccgcgcaca catcggatgg 7860 tggctcggcc ccaaccacgt gtgccgcacc gacagcgccc gactgacgcc actgcgctac 7920	cgaataattt tccatatagc	ctcggcgcgt cggactcgaa	tagttgatgt gggcg	ggcac 7560	
agccgcgacg acgcattggc aatgcggtac gtcgagcatt caccgcacgc gttgctcgga 7740 tctatcgtca tcgactgcga tcacgttgac gccgcgatgc gcgcattcga gcaaccatcc 7800 gaccatccgg cgccgaactg ggttgcacaa tcgccgtccg gccgcgcaca catcggatgg 7860 tggctcggcc ccaaccacgt gtgccgcacc gacagcgccc gactgacgcc actgcgctac 7920	agttgcccca tgaaatccgc	aacgggggggc gtgctgagcg	atcggcaatg ggcgg	atgcg 7620	
tetategtea tegaetgega teaegttgae geegegatge geegeattega geaaceatee 7800 gaecateegg egeegaaetg ggttgeaeaa tegeegteeg geegegeaea eateggatgg 7860 tggeteggee eeaaceaegt gtgeegeaee gaeagegeee gaetgaegee aetgegetae 7920	gtgttgcttc cgcaccggcc	gttcgcgacg aacaacctcc	aacgaggtca gtacc	ggatg 7680	
gaccatccgg cgccgaactg ggttgcacaa tcgccgtccg gccgcgcaca catcggatgg 7860 tggctcggcc ccaaccacgt gtgccgcacc gacagcgccc gactgacgcc actgcgctac 7920	agccgcgacg acgcattggc	aatgeggtae gtegageatt	caccgcacgc gttgc	tcgga 7740	
tggeteggee ceaaceaegt gtgeegeaec gaeagegeee gaetgaegee actgegetae 7920	tctatcgtca tcgactgcga	tcacgttgac gccgcgatgc	gcgcattcga gcaac	catcc 7800	
	gaccatccgg cgccgaactg	ggttgcacaa tcgccgtccg	gccgcgcaca catcg	gatgg 7860	
gcccaccgca tcgaaaccgg cctcaagatc agcgtcggcg gcgatttcgc gtatggcggg 7980	tggctcggcc ccaaccacgt	gtgccgcacc gacagcgccc	gactgacgcc actgo	gctac 7920	
	gcccaccgca tcgaaaccgg	cctcaagatc agcgtcggcg	gcgatttcgc gtatg	gcggg 7980	

		-		-
-cc	hnt	· i r	2114	ьd

				-concri	Iucu	
caactgacca	aaaacccgat	tcaccccgat	tgggagacga	tctacggccc	ggccaccccg	8040
tacacattgc	ggcagctggc	caccatccac	acaccccggc	agatgccgcg	tcggcccgat	8100
cgggccgtgg	gcctgggccg	caacgtcacc	atgttcgacg	ccacccggcg	atgggcatac	8160
ccgcagtggt	ggcaacaccg	aaacggaacc	ggccgcgact	gggaccatct	cgtcctgcag	8220
cactgccacg	ccgtcaacac	cgagttcacg	acaccactgc	cgttcaccga	agtacgcgcc	8280
accgcgcaat	ccatctccaa	atggatctgg	cgcaatttca	ccgaagaaca	gtaccgagcc	8340
cgacaagcgc	atctcggtca	aaaaggcggc	aaggcaacga	cactcgccaa	acaagaagcc	8400
gtccgaaaca	atgcaagaaa	gtacgacgaa	catacgatgc	gagaggcgat	tatctgatgg	8460
gcggagccaa	aaatccggtg	cgccgaaaga	tgacggcagc	agcagcagcc	gaaaaattcg	8520
gtgcctccac	tcgcacaatc	caacgcttgt	ttgctgagcc	gcgtgacgat	tacctcggcc	8580
gtgcgaaagc	tcgccgtgac	aaagctgtcg	agctgcggaa	gcaggggttg	aagtaccggg	8640
aaatcgccga	agcgatggaa	ctctcgaccg	ggatcgtcgg	ccgattactg	cacgacgccc	8700
gcaggcacgg	cgagatttca	gcggaggatc	tgtcggcgta	accaagtcag	cgggttgtcg	8760
ggttccggcc	ggcgctcggc	actcggaccg	gccggcggat	ggtgttctgc	ctctggcgca	8820
gcgtcagcta	ccgccgaagg	cctgtcatcg	accggcttcg	actgaagtat	gagcaacgtc	8880
acagcctgtg	attggatgat	ccgctcacgc	tcgaccgcta	cctgttcagc	tgccgcccgc	8940
tgggcatgag	caacggccaa	ctctcgttca	a			8971

1. A mutant of a microorganism of the genus *Rhodococcus* having a higher sensitivity to lysozyme than a wild-type microorganism of the genus *Rhodococcus*.

2. The microorganism of the genus *Rhodococcus* according to claim 1, wherein the microorganism of the genus *Rhodococcus* is *Rhodococcus* erythropolis.

3. The microorganism of the genus *Rhodococcus* according to claim 2, wherein the *Rhodococcus erythropolis* is *Rhodococcus erythropolis* strain L-65 (Accession No. FERM BP-8443) or *Rhodococcus erythropolis* strain L-88 (Accession No. FERM BP-8444).

4. A method of producing a protein comprising transforming a mutant of a microorganism of the genus *Rhodococcus* having a higher sensitivity to lysozyme than a wild-type microorganism of the genus *Rhodococcus* by a gene encoding an exogenous protein; expressing the gene; and treating the microorganism of the genus *Rhodococcus* with lysozyme, thereby extracting and recovering the protein.

5. The method of producing a protein according to claim 4, wherein the microorganism of the genus *Rhodococcus* is *Rhodococcus* erythropolis.

6. The method of producing a protein according to claim 5, wherein the *Rhodococcus erythropolis* is *Rhodococcus erythropolis* strain L-65 (Accession No. FERM BP-8443) or *Rhodococcus erythropolis* strain L-88 (Accession No. FERM BP-8444).

* * * * *